THE MICROSCOPIC DIAGNOSIS OF HUMAN MALARIA

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THE MICROSCOPIC DIAGNOSIS OF HUMAN MALARIA

II—A MORPHOLOGICAL STUDY OF THE ERYTHROCYTIC PARASITES

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PREFACE

Within a stone s throw of this Institute is the main government hospital of the State of Selangor, a large general hospital with some six hundred beds Here in the wards are represented most of the diseases of Malaya and here, by the continued courtesy and co-operation of the State Government the Institute has undertaken some of its most useful work—on beri beri scrub typhus typhoid fever dysentery leptospirosis and other diseases Here too have been centered the clinical studies on malaria which beginning with the work of William Fletcher on the alkaloids of cinchona have continued almost unbroken for the past twenty five years In this hospital and in the District Hospital Tampin where the Negri Sembilan State Government has conceded similar facilities some thousands of Asian patients have been treated for malaria under conditions appropriate for the experimental evaluation of new remedies Over the years this work has brought a growing knowledge of the malaria parasites prevalent in Malaya their form in thick and thin blood films and their numbers and clinical associations We have thought that this knowledge from its practical bearing on the problems of microscopic diagnosis and prognosis in malaria might be useful to colleagues in the hospitals, and in 1949 an account of the morphology of the Malayan parasites in thick blood films was issued from the Institute as Study No 23 with a suggestion that a companion volume on thin film diagnosis might appear at some later date With the publication of Study No 24 this suggestion now takes shape

The present study describes and illustrates the appearances in thin blood films of the four species of Plasmodium infecting man with particular emphasis on the forms prevalent in Malaya The drawings of the Malayan parasites are original they were made at the microscope mostly from material prepared in the course of the day by day routine work of the Malaria Research Division of the Institute

The earlier study excluded *Plasmodium ovale* a species not known to occur in Malaya a species moreover of which I have no first hand knowledge My good fortune in having been able to enlist the support as co-author of Mr P G Shute enables me in this later study not only to correct what I believe to have been a significant omission but to draw on a widened range of experience and clinical material

KUALA LUMPUR
December 1955

J W F

ACKNOWLEDGEMENTS

The parasitological material prepared in past years by the Malaria Research Division of this Institute and the clinical records of some thousands of patients who have been treated for malaria by the divisional staff in the ward of the government hospitals in Kuala Lumpur and Tampin provide an essential basis for this study We thank our colleagues at the Institute and particularly Dr T Wilson and Dr J F B Edeson the present malaria research officers for having placed this material so freely at our disposal and the medical officers of the hospitals for their co-operation

The library of the Institute is still depleted from the lo es of the war period and we have drawn heavily on sources of medical reference not available in Kuala Lumpur We thank the Librarian University of Malaya the Librarian Wellcome Historical Medical Museum, the Royal Society of Tropical Medicine and Hygiene Sir Gordon Covell Professor Raffa le Professor P C C Garnham Dr E Mosna and Dr E S Pampana for their substantial help in this direction and Sir Malcolm Watson for the loan of his original drawings of the developmental stages of falciparum gametocytes Text figures from various books and journals are reproduced by the kind permission of Dr M F Boyd (figs 10 and 19 from Malariology Saunders & Co pp 100[illegible] and 103[illegible]) the Royal Society of Tropical Medicine and Hygiene (fig [illegible]o from the Transactions of the Society Vol 4[illegible] p 135) the Editor Australian Journal of Experimental Biology and Medical Science (fig 23 from Vol 26 of the Journal p 515) and the Editor American Journal of Tropical Medicine and Hygiene (fig 15 from Vol 2 of the Journal p 958) Most of the drawing in plates I XXXV are original but we have redrawn for comparison a few figures from other sources These figures taken mainly from plates originally published in colour are reproduced by the kind permi sion of the editors from the journals— American Journal of Tropical Medicine and Hygiene Annals of Tropical Medicine and Parasitology Annales de l Institute Pasteur de Pari Bulletin de la Société Pathologie Exotique, East African Medical Journal Indian Journal of Medical Research Journal of Experimental Medicine Journal of the National Malaria Society Parasitology Rivista di Malariologia We have pleasure also in recording our thanks to Miss Elizabeth Cheek Biochemist of the Institute and Mrs Il e Tav Librarian for checking the text and references and to Mrs Olwen Price Mr A de Rozario and Mr Lit Kwai Loong for preparing the typescript

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CHAPTER I

A SHORT HISTORY OF MICROSCOPIC DIAGNOSIS IN HUMAN MALARIA

For many years I have held the opinion that the intermittent fevers are produced by parasites which renew the paroxysm by the act of their reproduction which occurs more or less rapidly according to their species This theory advanced in the early nineteenth century by Rasori expresses in more precise terms and with remarkable prescience an idea so commonly held in Italy that the fever producing parasites had received from the people a name—*serafici* or death producing Restated later by others in various ways though seldom so clearly as by Rasori this hypothesis aroused interest in the nature of the *serafici* and sundry algae vegetable spores or bacteria were held to cause malaria—none it seemed on better grounds than the *Bacillus malariae* of Klebs and Tomassi Crudeli Announced in 1879 and received with enthusiastic acclaim the discovery of this bacillus the last false scent on a hot trail misled for several years even the great Italians whose later work laid the enduring foundations of our present knowledge

The true parasite of malaria was almost certainly seen and passed by unrecognised in the mid nineteenth century Meckel in 1847 described pigment in round oval or spindle shaped protoplasmic masses which were undoubtedly malaria parasites and Virchow a year later pictured certain forms of the parasites but with no hint that they might be living organisms More than thirty years passed before Laveran with the leap of intuitive perception which was his great contribution to medicine announced their true nature

LAVERAN

Alphonse Laveran a French Colonial Surgeon in Algeria was working at the Military Hospital Constantine when he took upon himself the task of investigating the malaria fevers He saw pigmented crescent shaped bodies in the blood of persons suffering from fever and was still uncertain of their nature when the explosive activity of one of the bodies left no doubt that he was observing a live organism It is perhaps fitting that we should recall the circumstances of this momentous discovery The patient a young soldier of the 8th Squadron of Artillery stationed in a notoriously unhealthy barracks was admitted to the Constantine Hospital with fever on October 12th 1880 He was treated with quinine the fever yielded and he was discharged from hospital to be re admitted with a relapse and treated again with quinine On November 5th numerous crescent shaped and spherical pigmented bodies were seen in the blood For the events of November 6th we may perhaps quote Laveran s own words I had proceeded thus far with my researches and was still hesitating whether these elements were parasites when on November 6th 1880 on examining the pigmented spherical bodies I observed on the edge of several of these elements moveable filaments or flagella whose extremely rapid and varied movements left no doubt as to their nature

The pigmented spherical bodies with flagella were the male gametocytes of the malaria parasite which was later named *Plasmodium falciparum* The identity of the crescent shaped bodies is equally beyond doubt though it is not easy to identify some of the structures of which Laveran writes from his descriptions and rather crude drawings

This was Laveran s great discovery He saw the strange bodies in the blood of fever patients he recognised that they were live organisms and he described them so that others might follow His observations at the microscope were usually made with a dry objective on fresh unstained blood though he sometimes used the oil immersion lens He preferred daylight for artificial light with the Abbé condenser made the parasites too transparent.

Staining he sometimes used, though with obvious reluctance It is in the pure and fresh serum, he writes in the midst of the blood corpuscles that the parasites are best studied Thus, with means so imperfect did the microscopic diagnosis of malaria have its beginnings

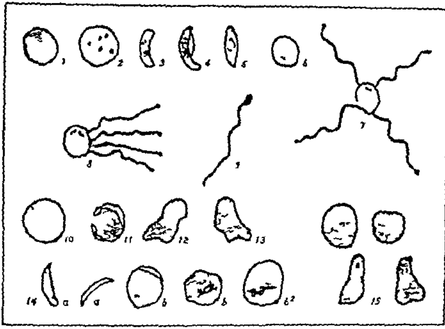


Fig 1—THE PARASITES OF MALARIA (after Laveran, 1881)

Laveran s announcement was received with scepticism and for several years more the bacillus of Klebs and Tomassi Crudeli held the field The young Italian school remained unconvinced even when Laveran came to Rome to demonstrate the parasites Marchiafava and Celli, whose researches were soon to soar beyond those of Laveran thought that the parasites were areas of degeneration in the red cells and only in 1884 when they began to examine fresh blood with a 1/12 immersion lens were they prepared to concede the accuracy of his observations How far the homogeneous immersion lens contributed to the advances of the next ten years we cannot know The formula for immersion lenses had been published in 1874 and in 1878 Carl Zeiss had begun to make them From Laveran s writings we are left to presume that his early work was done with dry objectives , the exacting observations from which the erythrocytic life cycle of the parasites was minutely defined by the later Italian researches had the immense advantages of the immersion system

GOLGI

Working in Algeria, and seeing parasites of three species in their various forms, Laveran sought with little success to interpret a confusing picture The organisms he thought were pleomorphic forms of a single species , he had no clear notion of their life cycle nor did he relate their growth to the clinical phenomena of infection The notable advances which clarified these obscurities we owe to Golgi Working in Padua where the malignant fevers were rare Golgi studied the parasites of the mild tertian and quartan fevers undistracted by

LAVERAN A (881) A communi n dd ed f om Con t ine on 25th Dec mbe 880 th Soci te Med cal des Hô p taux d Par d publ h d in h Bull tin f th S ci ty n 1881 (C ted by S que ra J H 1930 Alph nse La ran and hi w k *Keny E Af m d J* 6 319)
— (1891) *Palud m* The New Syd nham *Soci ty L ndon 1893*

the diverse forms of the malignant parasite He recognised that the parasite causing the quartan fevers could be distinguished by its constant form and by a constant 72 hour life cycle from the youngest amoeboid forms to the segmenting rosette and he observed that the parasites tend to reach maturity at the same time Then by comparing the stage of the cycle with the symptoms in the patient he established the important fact that the malaria paroxysm always coincides with the segmentation of a group of parasites Thus a single brood of the quartan organism causes a paroxysm every three days two broods two paroxysms in the same period and three broods three with patterns of temperature which are described as quartan double quartan or triple quartan (quotidian)

These highly significant researches begun in 1885 were succeeded by a series of observations equally remarkable on the parasites of the simple tertian fevers Here again Golgi was able to show that the tertian parasite has constant morphological features that it differs from the quartan organism in having a 48 hour cycle and that the cycle and the clinical features of the simple tertian fevers are interrelated

From Golgi s work it was clear that he recognised two kinds of malaria parasites possibly three for he hints that the crescent shaped bodies of Laveran might represent yet another kind More important still his work brought the observations made at the microscope into exact relation with the symptoms of the patient

MARCHIAFAVA

While Golgi was studying the mild tertian and quartan fevers in Padua Marchiafava with his colleagues Celli and Bignami were investigating the malignant summer autumn fevers of Rome Like Golgi they sought to harmonise what they saw at the microscope with their observations at the bedside but the Rome fevers were often fatal and they were able also to study the form and distribution of the parasites in the body after death Something of the enduring quality of their work between 1885 and 1894 is perhaps best conveyed by brief extracts from their writings

It is from the examination of the parasites that we can draw materials which enable us to form our prognosis of the dangerous fevers

The parasites present such special characteristics that a differential diagnosis may be made on the first resort to the microscope

We cannot recall a single case of malarial infection in which repeated and properly conducted examination of the blood did not reveal the presence of parasites

First and foremost in the cases which we have studied up to the present we see that malignancy coincides with this exceptionally abundant quantity of parasitic forms

Everyone knows who has studied these fevers that it always remains difficult to find forms of fission in the blood of the finger Indeed this is the reason why the reconstruction of the life cycle of this variety of haematozoon has been accomplished with such difficulty and it has only been successful when the examination of the blood in life has been combined with the study of the different viscera in cases of fatal malignancy

In the majority of the malignant infections an enormous invasion of parasites takes place, whereby the capillaries of certain organs e g the brain are so filled with amoebiferous red blood-corpuscles that often none but a few normal ones can be found

In the summer autumn fevers whilst a great portion of the development of the parasites especially segmentation does take place in the internal organs there occurs in a determinate

Marchiafava E and Bignami A On Summer Autumn Malarial Fevers The New Sydenham Society London 1894

relation with the course of the paroxysm of fever, an invasion of young amoebae into the circulating blood The presence of forms of advanced development and in a state of segmentation in the blood is a certain indication of gravity

It is not the crescent shaped body but the amoeba that has the greatest pathological significance—the amoeba which passes through a developmental cycle *with every paroxysm* of fever

There are certain instances of fatal malignant infection in which, although during life but few crescent shaped forms show themselves in the blood the autopsy reveals an extremely large number of them But they are not uniformly distributed in the vessels of each of the viscera a very large predominance is found in the bone marrow where they may be seen at all stages The spleen contains them in smaller numbers, while in the other organs they are very scanty

On the seventh or eighth day of the disease there are found in the blood extracted from the spleen and exceptionally in the blood of the finger (as in cases where the parasites are extremely numerous) endogenous pigmented corpuscles ovoid or spindle-shaped the evolution of which may be traced up to the adult crescent shaped form After the formation of the young crescent shaped forms has thus commenced on an average towards the seventh or eighth day of the disease their production continues with each successive paroxysm so that after a series of attack the blood contains an accumulation of crescent shaped forms

From Marchiafava s pen nearly forty years later came one of the finest accounts of the pernicious fevers ever written But he could add little to what he and his colleagues of the Italian school already knew in 1894 and little that is fundamental to our knowledge of the erythrocytic parasites has been added since

THE SPECIES OF MALARIA PARASITES

The early idea that all the malaria organisms are pleomorphic forms of a single species though held with all the weight of Laveran s authority became untenable when Golgi showed that the quartan and the simple tertian parasites are different from the malignant summer autumn parasites and from each other, and by the turn of the century three of the four species *we recognise to day had been generally accepted*

(*a*) The parasite of the quartan fevers with a 72 hour cycle, first seen and illustrated by Laveran, and recognised as a separate species by Golgi

(*b*) The parasite of the simple tertian fevers with a 48 hour cycle first recognised as a separate species by Golgi ,

(*c*) The parasites of the irregular summer autumn fevers with an undetermined cycle of 24 48 hours first seen and described by Laveran and recognised by *Marchiafava and Celli as the specific cause of the malignant fevers*

The fourth, also with a 48 hour cycle and causing a mild tertian fever was described by Stephens thirty years later

The malignant parasite—The cycle of the *parasite* of the dangerous summer autumn fevers was difficult to establish for the parasites, unlike those of the quartan and simple tertian fevers, usually recede to the inner organs when they are about half grown and the broods tend to overlap The patterns of temperature quotidian in some cases and tertian in others suggested that there might be two varieties *Marchiafava and Bignami took this view* Mannaberg thought that there might be a third distinguished by the absence of pigment. These refinements of distinction are now mainly of historical interest Few workers to-day

MARCHIAFAVA E (1931) Pernicious malaria *Amer J Hyg* 13 1 56

recognise more than one kind of malignant tertian parasite but fifty years ago when Hamilton Wright working at this Institute and accepting Mannaberg s classification made the first systematic study of the Malayan parasites the distinction found wide acceptance

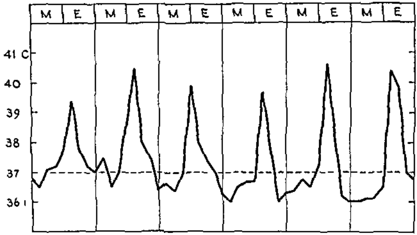


Fig 2—MALIGNANT QUOTIDIAN FEVER (after Marchiafava and Bignami 1894)

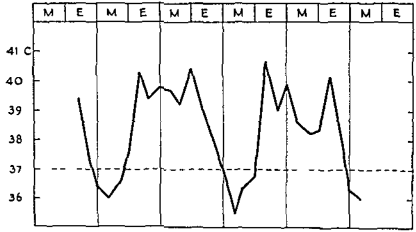


Fig 3—MALIGNANT TERTIAN FEVER (after Marchiafava and Bignami 1894)

We shall have occasion to discuss the variations in the malignant parasite in a later s ction but the early classifications may help to give historical perspective

Marchiafava and Bignami

(*a*) The quotidian parasite with a 24 hour cycle distinguished morphologically by the smaller size particularly of the mature segmenters may rarely segment before pigment appears tends to cause pernicious types of infection

MANNABER J Th *Mal ri f P* Th N w Syd nham Soc ty Lo d 894
WRIGHT H (90) Th Mal rial F rs f B t h Mal ya, *S udy N 1 Ins m d Re* F d d M l y States

(*b*) The malignant tertian parasite with a 48 hour cycle, a larger parasite at all stages, young forms tend to appear in the blood several hours after the beginning of the paroxysm much later than those of the quotidian organism

Mannaberg

(*a*) The pigmented quotidian parasite with a 24 hour cycle, smaller at all stages than the malignant tertian

(*b*) The unpigmented quotidian parasite, described by Mannaberg as one of the most beautiful discoveries in the field of malaria etiology, resembles the pigmented quotidian in all respects except in its failure to produce pigment during the asexual erythrocytic cycle

(*c*) The malignant tertian parasite with a 48 hour cycle, larger at all stages than the quotidian parasites more active, pigment formation delayed until the parasite is more than 24 hours old, febrile attack lasts for 24 40 hours

THE NAMES OF THE PARASITES

The early accounts of the malaria parasites were confused by an embarassing variety of names This confusion inevitable perhaps in the circumstances of the original discovery and the dawning knowledge of different species, has not yet entirely disappeared Laveran recognising malaria parasites of one kind only, and seeing a parallel in the active movements of the exflagellating forms, placed them in the genus *Oscillaria* created many years earlier to include various algae with animal like movements He named them *Oscillaria malariae* Four years later, in 1885, Marchiafava and Celli introduced the name *Plasmodium* for the young amoeba like parasites of the malignant fevers This name came to stay but it took root with a wider meaning to become the generic name for all the malaria parasites The main sources of confusion however, were still to come Grassi and Feletti in 1890 thought that there were parasites of two main kinds—those resembling amoebae which they named *Haemamoeba* and those with a crescent like form which they called *Laverania* With mistaken views on their mutual affinities they named five species of these two genera

(*a*) *Haemamoeba malariae* the parasite of the quartan fevers,

(*b*) *H vivax*, the parasite of the mild tertian fevers,

(*c*) *H praecox* the pigment forming parasite of the malignant fevers (crescent like forms excluded),

(*d*) *H immaculata* the parasite of the malignant fevers forming no pigment up to segmentation (crescent like forms excluded),

(*e*) *Laverania malariae* the crescent like parasite of the malignant fevers

This was confusion indeed for the crescents and the amoeboid forms of the malignant parasites appeared in separate genera, but at least it brought a clear recognition of the separate identity of the quartan and mild tertian parasites and gave them the specific names they bear to-day

The quartan and mild tertian parasites—The specific names *malariae* and *vivax* proposed by Grassi and Feletti for the parasites of the quartan and mild tertian fevers were not accepted forthwith and Schuffner in 1899 was still steering a course clear of the shoals of nomenclature when in his paper describing the stippling of the vivax infected cell he referred to the parasites simply as tertian and quartan But the names were open to little serious criticism and when Schaudinn in 1902 recommended the adoption of the single genus *Plasmodium* the species *malariae* and *vivax* were no longer a source of controversy Henceforth the names of the quartan and simple tertian parasites became *Plasmodium malariae* (Grassi and Feletti 1890) and *Plasmodium vivax* (Grassi and Feletti, 1890)

The malignant parasites—The naming of the malignant parasites was more difficult Laveran in 1881 gave the name *Oscillaria malariae* to parasites which from his descriptions were clearly tho e of the malignant fevers Marchiafava and Celli in 1885 introduced the name *Plasmodium* for the young amoebae of the same parasites Grassi and Feletti in 1890 placed the differing forms of the malignant parasites in two genera *Haemamoeba* and *Laverania* creating three species of these genera *praecox immaculata* and *malariae* and finally Welch in 1897 proposed the single name *falciparum* The rules of zoological nomenclature brought no lasting solution for these confusions nor could a solution be expected while the affinities of the parasites remained in doubt

We cannot in this glancing reference to the nomenclature of the parasites enter into the complexities with which the naming of the malignant parasite was beset The subject has been discussed by Christophers and Sinton (1938) and by Missiroli (1938) and the interested reader may turn to these authoritative sources of information Four generic names had been proposed for the parasites of the malignant fevers—*Oscillaria* (Laveran 1881) *Plasmodium* (Marchiafava and Celli 1885) *Haemamoeba* (Grassi and Feletti 1890) and *Laverania* (Grassi and Feletti 1890) Laveran s name *Oscillaria* was soon abandoned and *Haemamoeba* was equally short lived Maurer in an excellent description of the parasite published in 1902 called it *Laverania malariae* a name which Thomson and Woodcock in 1924 tried to revive but despite strong claims for recognition the name has now almost disappeared Blanchard in 1905 seeing no good reason for separating *Haemamoeba* (— Plasmodium) from *Laverania* placed all the human malaria parasites in the single genus *Plasmodium* His view was widely accepted and the name *Plasmodium* took firm root Some authorities think that this is unfortunate that the distinctive form and behaviour of the malignant parasites are grounds enough for admission to the separate genus *Laverania* and that in any event a name which honours Laveran would have been more happily chosen But the time for change has passed the genus *Laverania* could be raised again only at the price of confusions which few to-day would consider justified

No less confusing was the problem of species Is there but one kind of malignant parasite or are there two? This problem long debated and still not decisively settled turns mainly on the duration of the cycle in the blood Marchiafava and Celli recognised two kinds tertian and quotidian with blood cycles of 48 and 24 hours Mannaberg went even further by suggesting that there are two kinds of quotidian parasite one which produces pigment during schizogonic development and one which does not Grassi and Feletti gave to these parasites the names *Laverania malariae* (malignant tertian) *Haemamoeba* (= Plasmodium) *praecox* (pigmented quotidian) and *Haemamoeba immaculata* (unpigmented quotidian) Support for these distinctions came from the Far East where Craig in the Philippines and Wright in Malaya found malignant parasites which they believed to have a 24 hour cycle and from Ziemann who recognised an African variety with a 24 to 48 hour cycle We cannot altogether dismiss the minute and exacting observations of these early workers but the differences they describe have never been proved and most authorities to-day accept but one malignant parasite with a cycle of about 48 hours Blanchard in 1905 adopting the species created by Welch proposed that the parasite be called *Plasmodium falciparum* and this name despite all criticism became firmly established A few continental authorities however continue to use the names *P praecox* and *P immaculatum* and the name *Laverania malariae* has not disappeared from medical writings Whatever their claims for recognition these earlier names must yield to the pressure of custom and the over riding need for uniformity If and when a malignant parasite can be proved to have a 24 hour cycle then and then only will revision be necessary Meanwhile the name which has almost

Chr st her R [illegible] S [illegible] J A (1938) Th [illegible] M [illegible] A (1938) [illegible] R Parasit 2 [illegible]

universal approval is *Plasmodium falciparum* (Welch 1897) with the important synonyms *P immaculatum* (Grassi and Feletti 1892) *P praecox* (Blanchard 1900) and *Laverania malariae* (Grassi and Feletti, 1890)

A second tertian parasite—In Europe there is but one indigenous parasite of the mild tertian fevers , elsewhere particularly in Africa, these fevers may be caused by a parasite with differences in form which have seemed to justify the creation of a new species, the fourth of the malaria parasites of man Stephens described this parasite in 1922 from the blood of a patient coming from East Africa and called it *Plasmodium ovale* The name has been criticised on the grounds that Emin saw and described the same parasite in 1914 while a few authorities still regard it as an aberrant form of *P vivax* We must defer however, to the views of most authorities to day that *P ovale* is a valid species and that the full name should be *Plasmodium ovale* Stephens 1922

THE ROLE OF THE EXFLAGELLATING BODIES

The bodies which Laveran saw on the day of his great discovery were the exflagellating gametocytes of *Plasmodium falciparum* He saw the turbulence of the parasite, the pigment granules whirled about like a swarm of midges and the lashing of the filaments as they burst from the surface of the parasite and strove to break away , and time and time again he was able to convince his critics by this striking picture of vitality that the parasites were truly alive The filaments he regarded as the final and most perfect form of the parasite , of their true nature he had no clue They are found in all forms of malaria and the bodies from which they arise are unrelated to the symptoms of the patient , these facts were soon known to the Italian workers, but the biological role of the flagellating bodies remained a mystery—so much so that Grassi and Feletti actually placed the crescent bodies in the separate genus *Laverania* Manson the first to prove that a parasite—the filarial worm—might be carried to man by an insect thought that the flagellating bodies might play a part in the transmission of malaria Why he asked, should 'exflagellation occur only after the blood is drawn and never in the patient s body Might not the filaments continue the life cycle of the organism in some sort of insect most likely a mosquito ? Ross in 1895 following up Manson s hypothesis showed that the crescent like bodies would indeed exflagellate in the stomach of the mosquito but it was MacCallum who, in 1898 while still a student working at the Johns Hopkins Hospital, Baltimore, resolved the mystery of their biological role from a study of the blood of crows, infected with *Halteridium* a malaria-like parasite of birds Examining the infected blood on a slide he distinguished two forms of the mature organism an active form which produced flagella , and a passive form which did not He was then able to follow what seemed to be a process of sexual union This remarkable discovery so important for an understanding of the life cycle of the parasite so seldom described adequately in text books we may perhaps record in MacCallum s own words

> Both in the fresh and in the stained specimens of blood there can be seen differences which sharply distinguish two forms of the organisms The forms are identical in outline, but the protoplasm of one is granular and opaque as compared with the clear hyaline protoplasm of the other This distinction is well brought out in the stained specimen in which the hyaline form remains almost entirely unstained while the other takes on a well marked blue stain with methylene blue Of these it can be determined that the hyaline forms alone become flagellated These two forms, then become extruded alike from the corpuscle and lie free in the plasma but generally only a very short time elapses before the hyaline forms become flagellated according to the process so often and so accurately

MACCALLUM W G (1897) On the flagell t d form of the malarial p e Lanc t u 1240

described by workers on malaria The granular forms lie quiet beside the nuclei and *shadows of the red blood corpuscles which lately contained them* but are soon seen to be approached by the flagella which having torn themselves away from the hyaline organism from whose protoplasm they were formed struggle about among the corpuscles These flagella which so concentrate their protoplasm as to form a head swarm about the granular spheres and one of them *plunges its head into the sphere and finally wriggles its whole* body into that organism Immediately on the entrance of this flagellum it seems to become impossible that another should enter for they may be watched circling about vainly beating their heads against the organism The flagellum which has entered continues its activity for a few moments and the pigment of the organism is violently churned up Soon it becomes quiet again and remains so for from fifteen to twenty minutes when a conical process begins to appear at one side of the organism the pigment collecting mainly to the opposite side This process grows larger and the pigment becomes more and more condensed until finally we have a fusiform organism with a small spherical appendage crowded with pigment at one end This spindle shaped organism moves forward with a gliding motion sometimes turning at the same time on its long axis sometimes going through amoeboid contortions The idea suggests itself from their great power of penetration that they may be the resistant forms that escape from the body during life into the external world The whole process seems to be a sexual process analogous to the sexual process seen in the lower animals and plants which occurs under unfavourable conditions and results in the formation of a resistant spore

Later MacCallum saw part of this same process in the crescent like bodies of malignant malaria He saw the crescents become oval and then round and then after from twenty to twenty five minutes certain of the spherical forms became flagellated others and especially those in which the pigment formed a definite ring and was not diffused throughout the organisms remained quiet and did not become flagellated The flagella broke from the flagellated forms and struggled about among the corpuscles finally approaching the quiet spherical forms One of them entered agitating the pigment greatly sometimes spinning the ring about the rest were unable to enter but swarmed about beating their heads against the wall of the organism After the entrance of the flagellum the organism again became quiet and rather swollen but although in the two instances in which this process was traced the fertilised form was watched for a long time no form analogous to the vermiculus was seen.

The riddle of the flagellating and quiescent bodies was now solved They were the male and female sex cells or gametocytes parent cells of the male and female gametes destined to initiate a new cycle of development in the stomach of the mosquito The male gametes were the *flagella—a misnomer for true flagella are organs of locomotion—while the female gametes were* the quiescent rounded organisms which remained the same size as the gametocytes from which they arose

THE CYCLE COMPLETE

While the role of the exflagellating bodies was still in doubt Ross in India saw a malarial oocyst on the stomach wall of a mosquito *A stephensi* which had fed on a patient whose blood contained the crescent bodies The day was 20th August Mosquito Day 1897 one year later in July 1898 came his greatest discovery working with the malaria of birds he traced the development of the parasites in the mosquito from oocyst to sporozoite and proved that the bites of mosquitoes with sporozoites in the glands could infect healthy birds Bird to mosquito mosquito to bird the cycle was now complete Within a few months the Italian workers Grassi and Bastianelli described the full life cycle of the parasites of human malaria and Bignami

infected a man experimentally from the bite of an anopheles mosquito In parenthesis we may perhaps add that Ross s discovery was *confirmed in every important particular* by Daniels a former director of this Institute, who in December 1898 was sent to Calcutta for the purpose by the Malaria Commission of the Royal Society

REFINEMENTS OF MORPHOLOGICAL DIFFERENTIATION

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The organisms of true malaria all belong to the genus *Plasmodium* Four species of this genus are recognised as causes of malaria in man *Plasmodium falciparum* the parasite of malignant malaria *P vivax* the parasite of benign tertian malaria *P ovale* the parasite of an African mild tertian malaria and *P malariae* the parasite of quartan malaria

The parasites carried to man by the bites of mosquitoes of the genus Anopheles (Gk *anophelein*—hurtful) enter the deeper layers of the skin as slender lance like organisms called sporozoites (Gk. *sporos*—a seed *zoon*—a living animal) A few sporozoites may enter tissue cells near the site of the bite but many quickly find a way into the blood stream where they remain for about half an hour Then they disappear from the blood to enter the liver—and possibly other fixed tissues—where they develop in the parenchymal cells and grow to be large organisms from 25 μ to 40 μ in diameter These liver parasites are called schizonts (Gk *schizein*—to cleave *zoon*—a living animal) because they divide and multiply by a process of cleavage in the organism More fully they are known as *liver schizonts* to avoid confusion with analogous forms which appear later in the blood Within about a week from the time of infection each liver schizont has grown and divided to produce some hundreds of daughter cells or *merozoites* (Gk *meros*—a part *zoon*—a living animal) The ripe liver schizont bursts liberating a swarm of merozoites *liver merozoites* we may call them some of which enter the blood stream and invade the red blood corpuscles Since the liver infection precedes the invasion of the red blood cells the liver schizonts are *pre erythrocytic* they are also *exo-erythrocytic* for they grow outside the red cells—hence the terms pre-erythrocytic forms or exo erythrocytic forms

The first generation of liver schizonts apparently brings to an end the schizogonic development of *P falciparum* in the liver but it seems that *P vivax* and possibly *P ovale* and *P malariae* may re invade the parenchymal cells to begin a recurring liver cycle The names *primary* and *secondary exo-erythrocytic* forms distinguish between the first and later generations The secondary exo-erythrocytic forms of *P vivax* persisting in the liver have been held to be the cause of relapsing infection

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Stains of the Leishman type are not defined compounds for the ripening of methylene blue *produces a variable mixture of oxidation products* Giemsa in 1902 prepared a stain of more definite composition by mixing methylene blue with methylene azure in fixed proportions This mixture, dissolved in glycerine and methyl alcohol, is the stain which bears his name

The Romanowsky stains, and especially Leishman s and Giemsa s modifications, have made an immense contribution to malaria diagnosis Simple to use, and clear in their differentiation *in colour* of the structure of parasite and host cell they were adopted throughout the world , and today, as indeed for many years past, the routine examination of blood for malaria is based almost exclusively on films stained by one or other of the Romanowsky methods

MICROSCOPY

An outstanding event *in the later history of visual microscopy was the introduction in 1878* of the homogenous oil immersion lens Discounting the actual discovery of the parasite, made at about the same time, no event has contributed more to precision in malaria diagnosis The advances of the present century are minor by comparison We see today little more than Golgi would have seen had he the same stained material but we see the microscopic image more comfortably a little more clearly and in a better light Fluorite immersion lenses give us an improved colour resolution binocular microscopes bring us the illusion of binocular vision with the reality of a better view , and the inclined binocular leaves us at ease through prolonged sessions at the microscope Low-voltage concentrated filament lamps give us a constant and brilliant light of controllable intensity , and light filters transmitting a known band of the spectrum heighten the colour contrasts of our stained material Yet with all these aids we are sometimes tempted to wonder how many of us today, seeing the young unpigmented unstained amoebae of *P falciparum* as Marchiafava saw them, would feel competent on such evidence to diagnose malignant malaria with equal assurance and certainty

SUMMARY

(*a*) The discovery of the parasite *coinciding opportunely with the appearance* of the oil immersion lens brought a new precision into malaria diagnosis Recognition of the disease, no longer dependent on the vagaries of the clinical picture, could be made with certainty from an examination of the blood

(*b*) The unravelling of the erythrocytic cycle and the recognition of different species brought to microscopic diagnosis the immense advantage of microscopic prognosis for in the words of Marchiafava, the greatest danger may be diagnosed and foretold by examination of the blood

(*c*) Diagnosis from the examination of the live parasite yielding slowly to a faltering use of inferior stains, and finally to the superb Romanowsky stains, had given us by the turn of the century the main essentials of the methods we use today

(*d*) The refinements of diagnostic method and interpretation developed over the past fifty years are minor by comparison with the discoveries of the great masters of the past

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CHAPTER II

THE HOST AND THE RED CELL

Stains of the Leishman ty... produces a variable r... definite com... mi... ...propriate forms of ...and accurately ...ue technical ...ic training to ...

...ienters, the ...s begins again ...om 48 to 72 hours— *P malariae* Whether ...ot finally decided

...ic development They grow they ...blood stream at fairly regular intervals ...n in appearance grow to full size without (Gk *gamos*—marriage , *kytos*—a cell) the ...e early workers They play no part in the clinical ...ate passively in the blood stream awaiting transfer to

...e formation or *gametogony* (Gk *gamos*—marriage , *genesis*— ...point in the erythrocytic development of the organism. Thereafter ... n the stomach of a female anopheles mosquito where the gametocytes m... ...ss through a phase of nuclear re-organisation a ripening phase known as *matu*... ...male gametocyte by a process known somewhat inappropriately as exflagellation ...*flagellum*—a whip), forms and liberates half a dozen or so whip like bodies, which are the male gametes the female gametocyte becomes a female gamete The male gametes small and active, are called *microgametes* the females large circular, and passive are the *macrogametes* Maturation takes place in the mosquito stomach soon after the blood has been ingested The motile male gamete swims around and penetrates and enters the body of the female with fusion of the nuclei The fertilised female gamete now called a *zygote* (Gk *zygosis*—a joining) at first spherical and motionless lengthens becomes mobile and is then known as an *ookinete* (Gk *oon*—an egg , *kineticos*—to move) The ookinete or travelling egg makes a way through the stomach contents with worm like movements reaches the stomach wall penetrates the epithelial lining and settles down beneath the delicate outer membrane as a small encysted body now called an *oocyst* The oocyst grows the nucleus divides and finally the ripe cyst becomes a large spherical body up to 50μ or more in diameter packed tight with spores—the *sporozoites* The cyst bursts, liberating the sporozoites into the body cavity of the mosquito whence they become diffused through the tissues and invade the salivary glands ready for transfer once again to a human host The process of sexual development in the mosquito beginning with the maturation of the gametocytes and ending with the production of sporozoites is known as sporogony (Gk *sporos*—a seed *genesis*—production)

THE ERYTHROCYTIC PARASITE

Numerous researches concerning the intimate structure of the malarial parasites have been made but the results are unfortunately as yet rather indefinite Thayer makes this

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In ... tricet ... ool gical sen e th par ... es whi h multiply by schiz gony in the blood cell ... blood chizon from th momen of the first ttachm t ... d o tinental writ rs usually g... th ... rm th wid m ru g B t th you g undivided p... tes ha ... u kn wn des ... y ... th y may b come the schizonts or game ocytes ... d morph l gical d ff ... tiation seldom pos bl unt l th y are fai ly dva ced in growth It ... h nce ... ient t es ... th t rm hi ... t for th paras which has d cl red t ld ... t y by ... plitting f the nucleu and to peak f th young undiff re ti d para t as t oph ... e

THAYER W S *Le tur ... th Mal ri l Fever* Henry Kimp on Lond n 1900

statement in his excellent lectures on the malarial fevers To-day fifty years later we are not very much wiser We still do not know the intimate structure of the malaria organisms nor do we know for certain where they lie in relation to the host red cells

Four structural features may be identified in the Romanowsky stained malaria parasite at some stage or another during development—chromatin and cytoplasm in every parasite pigment in the older parasites and in the young parasites an unstained area usually thought to be a vacuole Chromatin (Gk *chroma*—colour) is the substance in the nucleus of the organism which takes the red of the Romanowsky stains and gives to the stained nucleus its characteristic colour cytoplasm (Gk *kytos*—a cell *plasma*—something moulded or formed) is the blue stained body substance of the parasite pigment is the non staining yellowish granular material within the cytoplasm formed from haemoglobin as a by product of the metabolism of the organism and the vacuole or shall we say the presumptive vacuole is the clear unstained area in the trophozoite which gives it a ring like appearance Morphological description is based mainly on the form and relative arrangements of these four structural elements

The merozoite—The smallest parasite is the merozoite freshly liberated from the mature segmenter The appearance is that of a small rounded nucleus closely invested with cytoplasm the whole giving the impression of a compact spherule from 1μ to 2μ in diameter Sometimes there is a small space between the nuclear chromatin and the cytoplasm the beginnings of the vacuole

The trophozoite—*Once attached to a host red cell the merozoite becomes a trophozoite The vacuole enlarges and the stained organism takes on the familiar form of a signet ring The appearance is that of a ring but the reality behind the appearance is not fully known* The usual explanation is that the ring is an optical section of a stretched and flattened vesicle The protoplasmic wall of the vesicle has a varying thickness somewhere in its thinner part lies the nucleus which depending on the orientation of the organism appears either on the ring itself giving the signet ring appearance or within the vacuole Mary Lawson however from her studies of the structure of the young parasite over many years comes to a very different conclusion The ring she thinks is a linear parasite curled around a raised mound on the red cell surface The merozoite having attached itself to the cell pushes out minute cytoplasmic processes which spread over the surface to meet again forming a ring into which flows most of the cytoplasm of the organism Enclosed in the ring is a part of the red cell which is squeezed and heaped up as a corpuscular mound (plate I) This mound whence the parasite first derives its food becomes pale from the loss of haemoglobin the apparent vacuole of the organism is thus nothing more than a pallid area of the red cell surface Two parasites may be coiled around a single mound hence she suggests the double nucleus often seen in a single falciparum ring

Though based on careful observations and supported by hundreds of excellent photomicrographs the views of Mary Lawson have not been generally accepted Nor yet have they been disproved Where the truth lies we do not know maybe we shall never know until more refined methods of examination are available to us

Vesicular nucleus vacuole, or corpuscular mound—*The non staining area within the ring of the trophozoite has been variously described as a vesicular nucleus a digestive* vacuole and as an area of red cell surface enclosed by the pseudopodia of the organism Most of the early workers recognised a large bladder like nucleus with a small deeply staining nucleolus Romanowsky Grassi Feletti and Mannaberg held this view Bignami on the other hand regarded the small stained body as the true nucleus and the unstained area as a nutritive vacuole

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La s w Mas R (9 9) J 9 M d 23 63 17 344 19 45 27 739 29 36 5 39

A third view that the unstained area is merely a part of the red cell surface enclosed by the parasite, we have already mentioned Here we must leave the problem To day, Bignami s view seems to hold the field, and the varied appearances of the trophozoite are interpreted from the concept of a vesicular organism with a large vacuole, thin cytoplasmic walls, and a small deeply staining nucleus lying within these walls and appearing in optical section on the edge or within the vacuole, depending on the way the organism is lying The vacuole is a characteristic mainly of the young trophozoite, with advancing growth the cytoplasm closes in and the vacuole finally disappears

The nucleus—The nucleus of the malaria organism is the body which takes the red of the Romanowsky stains Seen in Romanowsky stained films the nucleus appears dense and structureless, for the chromatic material or chromatin, stains so deeply that the finer detail is lost Romanowsky speaks of a chromatin network ' implying that the chromatin is distributed throughout the nucleus as a reticulum Thompson (1932), obtaining a clearer picture by dissolving away the pigment from the organism and staining with haematoxylin, believed the nucleus of the falciparum trophozoite to be vesicular with the chromatin concentrated in a central karyosome (plate II) Boyd (1935) similarly thought the nucleus of *P vivax* to have a vesicular form with a distinction in lightly stained films between a central condensation of chromatin granules or karyosome, and a narrow clear space between the karyosome and a presumed limiting membrane Wolcott (1954), however, found no evidence of a vesicular structure in the non dividing nuclei of vivax trophozoites, the nucleus, he suggests, consists essentially of two coiled and condensed chromosomes

In Romanowsky stained films the nucleus of the trophozoite usually appears as a small round bead or blob but wide variations in form are not unusual particularly in films which are dried so quickly that the organism has no time to contract to its resting form The nucleus may be oval or linear shaped like a dumb-bell or curled like a horse hoe around the vacuole split into two or three pieces or drawn into bizarre linear patterns (plate VII) The common bead or blob appearance may perhaps give a false impression of the living structure We may conceive the nucleus in life to be in a semi fluid state, flowing around the vacuole with the amoeboid movements of the organism and contracting to a rounded solid looking blob only when disturbed or resting Granted the assumption that the nucleus in life may be stretched in tenuous strands with the amoeboid activity of the organism we may readily imagine the varied forms it would take when pushed and torn across a glass slide as the blood is spread, and prematurely fixed in a quickly dried film

The nucleus of the gametocyte has been studied by Thompson (193-) who interprets the appearances in films stained with haematoxylin as evidence of a vesicular structure, with a central karyosome, a clear surrounding zone and minute chromatin granules around the periphery In the male crescent he traces the division of the nucleus and the early formation of nuclei of the future gametes (plate II)

Schizogony and binary fission—Schizogony is a reproductive process leading to the formation of spore like bodies, or merozoites The nucleus divides into daughter nuclei which again divide and re divide until finally there are many nuclei in a single mass of cytoplasm Merozoites are formed when these nuclei acquire an independent cytoplasmic covering and separate to become individuals The process seems to be a modified form of mitosis, involving the division and separation of chromosomes Binary fission on the other hand is a primitive

THOMSON J G (1932) S m observati n th n l ar structure of th malignant tertian malari l p s te (*Plasmodium f lciparum*) *J t p M d (Hyg)* 3 1

BOYD M F (1935) On the schizogon us cycl f *Pl mod m vi ax* Gras and F l tti *Amer J t op M d* 15 605

WOLCOTT G B (1954) Nucl ar structure and divi on in th malaria paras te *Plasmodium vivax* *J Morph* 94 333

amitotic form of division from which two complete daughter organisms arise usually of the same size The amoebae divide in this way The body lengthens a constriction appears in the middle and the organism separates into two parts Schizogony is the normal method of division of the malaria organism binary fission a common method of reproduction among the protozoa has been sugg sted as an explanation for certain morphological peculiarities in the trophozoites of *P falciparum* and *P vivax*

Binary fission has been held to account for the curious pairing one sometimes sees in young trophozoites for the doubling of chromatin in the ring and for the multiple infection of cells which occurs so often in falciparum infections (Hingst 1934 Beach 1936 Yorke *et al* 19.9) Those who hold this view are usually prepared to accept schizogony as the main reproductive method but suggest that a direct division of the ring form into two four, or even eight daughter rings may sometimes come first We cannot dismiss this view but the morphological evidence in favour of schizogony as by far the most important if not the only method of reproduction is overwhelming and the appearances attributed to binary fission can usually be explained in other ways Chalmers and Archibald (19.0) reporting appearances in *P vivax* which suggest binary fission state their view that the whole process is a throw back to a method which may have been useful to some ancestor of the malarial parasites but which to day is entirely without practical importance and is rarely seen Few perhaps will contest this view

The schizont—Though in a strict zoological sense the asexual malaria parasite is a trophozoite throughout its entire period of growth regardless of the nuclear condition it is convenient to reserve the term for the young organism with an undivided nucleus, and to describe it as a schizont from the time of the first nuclear division Double or triple chromatin dots in the ring forms are not usually regarded as evidence of true nuclear division Beginning when the organism is rather more than half grown or even approaching full size schizogony continues until with the formation of fully differentiated merozoites segmentation is complete We may visualise the mature segmenters as rounded disc like bodies flattened by contact with the surface of the host red cell or if inside the cell by conformity with its structure Careful focusing of the upper and lower surface in a stained film reveals their depth to be no more than 1 2μ against a width of 5μ to 10μ depending on the species Though often smaller than the host cell the segmenters may completely fill the cell very rarely do they project beyond the edge a fact which has been held to show that they lie within rather than upon the cell

The gametocytes—Certain trophozoites at first undifferentiated in appearance are destined to become gametocytes They may be recognised at an early stage of growth, though with no great assurance by their compact form and with certainty at a later stage by the attainment of full size without nuclear division The full grown gametocytes of *P vivax* *P ovale* and *P malariae* are round or oval bodies flattened against or within the host cell Like the segmenters they do not project beyond the edge of the cell though they often occupy its whole surface The mature gametocytes of *P falciparum* the familiar crescents on the other hand are sausage shaped bodies—though they are called crescents they are seldom crescentic except when young—with poles which appear to project beyond the bounds of the host cell hence the suggestion that they lie on the outside Mary Lawson suggests that the crescent attaches itself to the red cell as a caterpillar wraps itself round a strawberry and if we grant the caterpillar to be a little flattened the simile is possibly apt The curved form we may conceive

Hingst H E (1934) *Plasmodium falciparum* W l B 1697 Does direct division of the parasite precede schizogony? *Amer J trop Med* 14 325

Beach T ... V (1936) Evidence of binary fission of ring forms in *Plasmodium vivax* Grassi and Feletti *Amer J trop Med* 16 147

Yorke W, Macfie J W S and Owen D U (1929) Observations on fission ... of blackwater fever *Trans R Soc trop Med Hyg* 23 335

Chalmers A J and Archibald R G (1920) The ... phase of *Plasmodium vivax* (Grassi and Feletti 1890) *J trop Med Hyg* 23 33

to be due to the drag of the adherent red cell as the crescent lengthens, or if it lies within the cell to the pull on the poles which the tensions in the cell might be expected to impose Supporting this view is the fact that young crescents freed by haemolysis from the host cells in thick blood films are often straight

With maturation the gametocyte ceases to be erythrocytic but the maturation process may be completed on a glass slide and we may perhaps pursue this cursory sketch of the structure of the organism to the point at which maturation is complete Maturation is the nuclear re organisation of the gametocytes which leads to the formation of male (micro) and female (macro-) gametes, ripe for fusion The process never begins until the blood has left the human host, normally it occurs in the mid gut of the mosquito though it may be seen in fresh blood examined under a coverslip It is presumed that with maturation there is a nuclear division in the gametocytes analogous to the reduction division or meiosis of the metazoa whereby the chromosomes in the sex cells are halved in number Mary MacDougall (1947) has shown that there are definite chromosomes in *Plasmodium* and Thomson and Woodcock (192_) illustrate appearances which they interpret as polar bodies small deeply staining masses of nuclear material extruded from the gametocyte after the reduction division (plates II and XVI)

The pigment—The yellowish pigment of the malaria parasites was known long before the parasites themselves A product of the metabolism of the parasite pigment is formed in the cytoplasm of the organism from the haemoglobin of the host red cell According to Brumpt the organism splits up the haemoglobin by means of proteolytic enzymes lives on the globin and discards the heme as a pigmented waste product This pigment known as haemozoin contains iron and is probably the same as haematin Minute granules of pigment begin to appear in the cytoplasm of the organism at the later trophozoite stage, thereafter they increase in number and size assuming a form and distribution which is characteristic of the species of the parasite and the stage of growth The varied patterns of the pigment we shall have occasion to discuss in later chapters

THE HOST CELL

Though the small round globules in the blood of man were described by the Dutch microscopist Leeuwenhoek in 1673 their intimate structure is still not fully known Is the red blood corpuscle a sac containing haemoglobin as a fluid colloid or is it a dense body with a thick wall as recent studies with the electron microscope seem to suggest? We know the cell to be a highly elastic structure with the shape of a biconcave disc that it contains haemoglobin and that the haemoglobin is held in such a form that it does not escape when the cell is divided From the physical properties of the cell we infer that the interior is filled with haemoglobin held as a fluid colloid in an elastic stroma with a surrounding membrane formed by a condensation of the lipoids and protein of the stroma This concept lies behind most of our views on the relation of the malaria organism to its host cell The organism adheres to the thin envelope and either remains there or penetrates to the fluid interior with the envelope closing in behind Maybe this is the true picture maybe not for recent studies of the red cell suggest the possibility of a different relationship Bernhard (1952) from an examination of thin sections with the electron microscope suggests that the intact red cell has a dense internal structure with a striking orderliness in its architecture and that a spongy network with a well defined membrane only appears when haemolysis sets in while the studies of Mitchison (1950) suggest that the

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M cDOUGAL MARY S (947) Cyt tological tudies f *Pl m d m* the mal gamete *J nat M l Soc* 6 9
THOMSON J D AND WOODCOCK H M Th P es f M l ria Chap er 69 *The P ctic J Med he T p* Ed Byam W d A h bald R G Henry F wd d Hodd d S oughto Lo d n 19
B UMPT E Th h m n paras es f th g nus *Pl mod um* Chap er 4 n *Malari l gy* Ed M F B yd W B S unders Company Philad lphi d Lo don 949
BE NHARD W (95) El tr n micr pe tudies on thin ctions of human ryth ocyt *N ur Lo d 170* 359
MITCH SON *J M* (1950) Thickn s and tructur *f th m mbran f the human d ll ghost. N ur Lond 166* 347

cell may consist largely of a dense membrane so thick that the inner surfaces touch in the middle of the disc

Whatever may be the intimate relations of parasite and host cell there are visible changes in the infected cell which are very useful in malaria diagnosis—changes in size in colour in configuration and in the apparent texture of the surface

Changes in size— In the spring tertian the corpuscle swells and grows pale rapidly while in the other tertian it tends to shrink and shrivel up The red globules which contain the quartan amoebae remain of normal size and appearance Marchiafava and Bignami recognising specific differences in the size of infected cells describe in these words the enlarged cell containing *P vivax* the shrinkage of the host cell due to *P falciparum* and the absence of change in the cell containing *P malariae* We can add little to these observations—*P vivax* enlarges the host cell, sometimes greatly *P ovale* produces a moderate enlargement *P malariae* leaves the cell unchanged These changes are extraordinarily constant and very useful in species diagnosis But we cannot accept the shrinkage of the falciparum infected cell without qualification The Italian workers speak of cells which shrivel to a third of their normal size shrinkage to this degree is seldom seen in cells containing *P falciparum* of Malayan origin The occasional cell may be dark and shrunken but as a rule the falciparum infected cell like the cell containing the quartan parasite remains the same size

There remains the grotesque enlargement sometimes found in red cells containing malaria parasites of any species sometimes indeed in cells which are not infected at all the great swollen degenerate cells which finally burst to produce the so called demi lune appearance This enlargement has no significance in malaria diagnosis (plate XXXIV)

Changes in shape—Variations in the shape of uninfected red corpuscles are quite common particularly in the anaemias but there are other changes due to the presence of the malaria parasite which are sometimes distinctive enough to be useful in diagnosis The enlarged host cell of *P vivax* becomes angular when it presses on neighbouring cells and the ovale infected cell tends not only to be oval but to have a fimbriated end especially when quickly dried in very thin films Crenation in cells containing *P falciparum* is fairly common even when the uninfected cells in the same microscopic fields are free from this spiky distortion in the cell margin while the young crescent stretches the cell to an oval form as it grows and lengthens

Changes in colour—The early writings refer to cells which take on the colour of old brass when infected with the malignant parasite—the globuli rossi ottenati of the Italian workers or the brassy corpuscles of the text books They refer of course to appearances in fresh blood for the falciparum infected cell in stained films in no way resembles old brass What appears to correspond to this change in stained films is the purplish tinge described and illustrated by Maurer (1902) and by Thomson (1924) in cells containing large falciparum rings This change is common but not invariable in cells containing *P falciparum* of Malayan origin A similar purple tinge possibly due to a selective invasion of immature basophilic erythrocytes is sometimes seen in cells infected with young vivax trophozoites (plate XLIX) The vivax infected cell seen in fresh blood is pale from the loss of haemoglobin but this change masked perhaps by the stippling is seldom obvious in the stained film

Changes in outline—A darkening and an apparent thickening of the rim of cells containing large falciparum rings was first illustrated by Maurer (190_) The occasional occurrence of crenation in falciparum infected cells and of fimbriation in the margin of cells containing *P ovale* has already been mentioned

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THOMSON J G (9 4) R search Bl kwa F in South rn Rhodes R h M mo l I Lond S hool f T op l M d p 35

CHAPTER III

PLASMODIUM FALCIPARUM (WELCH 1897)

NAMES AND SYNONYMS

Plasmodium falciparum is the name generally accepted for the parasite of the malignant malaria fevers The important synonyms are *Laverania malariae* (Grassi and Feletti 1890) *P praecox* (Blanchard 1900), and *P immaculatum* (Grassi and Feletti 1892) Other names with a loose clinical connotation are the subtertian and the malignant tertian parasite

The disease in man to which *P falciparum* gives rise has been variously called aestivo-autumnal or summer autumn fever, subtertian malaria malignant tertian malaria pernicious malaria and tropical malaria These names express particular attributes of the disease, a summer autumn incidence in some parts of the world frequent malignancy fevers which are common in the tropics and are sometimes tertian in type but these attributes are inconstant, and it is usual to-day to call the disease by the specific name of the parasite The more acceptable name *falciparum malaria* is free from ambiguity and universally valid

BEHAVIOUR

Plasmodium falciparum has important peculiarities of behaviour which mark it sharply from the other species infecting man The organism has a high rate of multiplication speedily attaining a density which may endanger the life of the host Segmentation moreover takes place in the capillaries of the inner organs, seldom in the peripheral blood, a hidden segmentation which, occurring in vital organs like the brain gives to the organism a pernicious character These two attributes go far to explain the malignancy of the organism in the human host Again the schizogonic forms present in the body at any one time tend to be of mixed phase they seldom march together in step but segment in successive broods at irregular times From this unsynchronised development derive the varied patterns of the temperature in the falciparum attack

HOST AND HABITAT

Parasites closely resembling *P falciparum* have been found in chimpanzees and gorillas but they are not the same and although *P falciparum* has been transmitted in the laboratory to infant howler monkeys of the genus *Alouatta* (Taliaferro *et al*, 1934) there is good reason to believe that in nature the only vertebrate host is man

The organism normally enters the body of man from the bites of infected anopheline mosquitoes The sporozoites, end products of sporogony in the mosquito enter the skin and are carried in the blood stream to the liver where they settle in the parenchymal cells, here they grow and multiply for six or seven days So far as we know, this pre-erythrocytic growth takes place only in the liver parenchyma, but the chance that other tissues may be involved has not been decisively excluded For the host the initial liver invasion is symptomless The liver schizonts ripen they produce merozoites which enter the blood stream to attack the red blood corpuscles and the exo erythrocytic phase of the organism comes to an end Henceforth again so far as we know, the growth of the organism in the human host is entirely endo-erythrocytic We shall see later that this transience in the liver stage in *P falciparum* is not shared by certain other species infecting man

GEOGRAPHICAL RANGE

The survival of *P falciparum* is broadly determined by temperature, and the pattern of world distribution is related to the mean summer isotherm The organism requires a sustained

TALIAFERRO W H ND TALIAFERRO LUCY G (1934) The tra mi on of *Plasmod um f lcip rum* to the howl r m nkey *Aloua* sp I G neral nature of the inf ti ns and morph logy of th par es *Amer J Hyg* 19 318

temperature of at least 20 C (70 F) to complete development in the mosquito and it thrives best at a mean temperature approaching 30 C (86 F) The theoretical frontier of falciparum malaria is hence the 20 C summer isotherm though as a rule the organism fades out well within this limit These conditions are common in hot countries *P falciparum* is hence distributed widely and often intensively throughout the tropics In sub-tropical regions the organism thrives only when the summers are long and hot infections are seasonal and the organism maintains a more precarious hold The northern limits include the Mediterranean Asia Minor South China and the Southern United States South of the equator in the sub tropics and beyond the organism has a more restricted distribution due to the smaller land masses, the scattered population and the lack of vectors

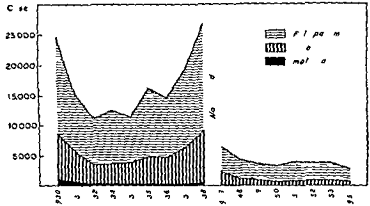


Fig 4—THE INCIDENCE AND PROPORTIONS OF THE FALCIPARUM VIVAX AND MALARIAE INFECTIONS TREATED IN THE GOVERNMENT HOSPITALS OF THE MALAY STATES OF SELANGOR PERAK NEGRI SEMBILAN AND PAHANG 1930–1954

Within the general pattern of distribution imposed by temperature there are local peculiarities of great variety and complexity The organism has been found beyond the limits of the summer isotherm of 20 C in the far north of Russia for example and in Kenya at a height of 2 600m (8 500 feet) with a mean temperature below 15 C (60 F) Here the indoor habit of the vectors seems to afford an explanation. In many Pacific islands within the usual limits of latitude and temperature the organism is absent owing to the lack of an efficient vector

Throughout the Malay peninsula *P falciparum* is the most common of the malaria parasites though there are wide variations in species distribution in particular areas The proportion of falciparum infections to vivax and quartan infections in persons admitted to Government hospitals for malaria over the past twenty five years is shown graphically in figure 4 The only significant change has been the marked drop in the proportion of quartan infections (*see* also p 68)

SCHIZOGONIC CYCLE IN THE RED BLOOD CORPUSCLES

The symptoms of malaria are due primarily to the parasites which multiply by schizogony in the red blood corpuscles Microscopic diagnosis depends mainly on the recognition of these schizogonic blood forms and microscopic prognosis is based on their numbers and stage of development

HACKETT L. W D tribut f Malari Ch pt 8 in M lari l'gy Ed M F Boyd W B Saunders Co London and Philad lphi 1949

The invasion of the blood occurs while malaria infection is still silent The parasites are too few to evoke a clinical response in the host but they multiply some ten to twenty fold at each forty eight hour cycle and despite heavy losses from the destruction of merozoites by the tissue defences, the numbers mount rapidly to the point at which the host reacts with fever The interval from the initial inoculation with the organism to the onset of fever is the *incubation period* sometimes called the *intrinsic incubation period*, in distinction from the *extrinsic incubation period* in the mosquito The parasite concentration in the peripheral blood at the time the host first reacts with fever of 100 F or more is the *pyrogenic or fever threshold* The period from inoculation to the first microscopic detection of parasites in blood films is the *prepatent period*

Incubation period—No reliable information on the incubation period of falciparum malaria in South East Asia is known to us Calculated from the time of infecting bites or from the time of deliberate inoculation with sporozoites or with infected blood, this period is usually determined from observations on experimentally induced malaria An incubation period between 10 and 14 days is usual in sporozoite induced infections Kitchen (1949) found the incubation

Table 1—INCUBATION PERIODS IN NATURALLY INDUCED FALCIPARUM MALARIA (data from Kitchen 1949 and Shute 1955)

Incubation in days	7 −	7	8	9	10	11	12	13	14	15	16	17	17+	Total
Infections (Kitchen)	—	3	3	4	18	9	73	18	48	17	13	4	10	220
— (Shute)	15	22	13	16	28	12	18	11	6	2	3	—	—	146

period in 220 infections naturally induced with six strains of *P falciparum* to lie as a rule between the twelfth and fourteenth day, in 146 infections induced with strains from West Africa Rome and Roumania and observed at Horton the incubation period was somewhat shorter usually falling between the seventh and twelfth day (table 1)

Prepatent period—Like the incubation period, the prepatent period is seldom known except in experimental infections and in South East Asia there are no reliable data Kitchen in America found that parasites were usually demonstrable in thick blood films before the host reacted with fever in a series of 2_0 infections, parasites were detected in 204 either before the first rise of temperature or on the same day The mean prepatent period was 11 days, 2 days less than the mean incubation period When diagnosis is made from thin blood films the prepatent period is, of course somewhat longer Shute, in sporozoite induced infections with falciparum strains from Rome and Sardinia, seldom found parasites in thin films until the second day of fever

Fever threshold—The fever threshold may be defined as the concentration of parasites in the peripheral blood at the onset of fever This concentration is no more than a rough measure of the density of parasites in the body as a whole the only true indication of the weight of infection necessary to evoke a febrile response *P falciparum* segments in the internal organs and the peripheral and deep concentrations do not necessarily run parallel We use the peripheral concentration for want of a truer index as it is the only one we can measure easily

The fever threshold in falciparum infections tends to be rather high Kitchen (1949) giving a mean threshold of 10 000 parasites per c mm for a series of 50 patients infected with the Costa strain mentions that the threshold may sometimes be as high as 95 000 Differences in the threshold are related to the tolerance of the host and high values are not unusual in secondary attacks when host immunity comes into play

KITCHEN S F Chapt 40 in *M l n l gy* Ed M F Boyd W B S und rs Co Phil d lphia and London 949

Microscopic threshold—The level of infection at which parasites are first demonstrable by the microscopic examination of blood films is known as the microscopic threshold Varying with the method of examination the experience of the observer and the number of fields examined the microscopic threshold is likely to be about 200 parasites per c mm of blood when 100 fields are examined by a good thin film method This means that one parasite will be seen in an average of 100 fields when the parasite density is .00 per c mm it assumes also that the first parasite seen will be recognised

Invasion of the red blood corpuscles—*Plasmodium falciparum* is seldom seen in the peripheral blood at the beginning of the schizogonic cycle The organism segments deep within the body and it is within the capillaries of the inner organs that the merozoites liberated from the mature schizonts attack the red blood corpuscles With rare exceptions the parasites appear in the peripheral blood as young trophozoites already several hours old Characteristically they are then minute rings 2 μ or less in diameter lying on the surface of the corpuscle or flattened against the margin Their varied patterns at this stage are illustrated in plates IV and V which are drawn from Malayan material

The rings enlarge the cytoplasm and chromatin increase in amount and within the next 18 to 24 hours the organisms treble in size (plate VI) Meanwhile the vacuole has become relatively small pigment has appeared as minute granules in the cytoplasm and the host cells have acquired the changes which appear in well stained films as Maurer s spots (plate VIII) This is the stage at which the parasites some 18 to 30 hours old normally recede to the deep organs and disappear from the peripheral circulation The invasion of the peripheral blood stream is hence limited to about half the schizogonic cycle and the forms seen in the blood with exceptions we shall consider later are trophozoites varying from . 1 to 4 or 5 μ in diameter

Recession of the ring forms—Under normal circumstances *P falciparum* disappears from the peripheral blood while still at a ring stage First observed by the early Italian workers this peculiarity is an important reason for the malignancy of the organism The parasites leave the peripheral blood to stagnate in the capillaries of the vital organs Recession begins as a rule when the rings are fairly large and pigment granules have appeared in the cytoplasm but it may begin earlier while the organism is still unpigmented and comparatively small or later when the ring appearance has passed and the organism is compact from the loss of the vacuole or still later though rarely when the nuclear chromatin has begun to divide

Marchiafava and Bignami explain the retention of the growing parasites in the de p organs as an expression of two factors—the greater circulatory resistance in the fine capillary network of organs like the brain and the peculiar resistance of the falciparum infected cell to a free flow in the minute vessels The observations of Thomson and McLellan (1912) on *P falciparum* in cultures seem to show why the infected cells do not circulate freely but stagnate in the capillaries When the parasites are about half grown the host cells develop a peculiar stickiness they adhere to the large mononuclear cells and to each other forming clumps which we may presume become impacted as emboli in the finer vessels

Small and large rings—The size of the smallest ring forms of *P falciparum* in blood films is related to the stage of the schizogonic cycle at which they appear in the peripheral circulation the size of the largest to the stage at which they recede to the inner organs At any one time in the course of an attack the rings may be so small that four or five could invade a cell without touching each other or so large that two rings would inevitably be in contact They may be at any stage in between though they are usually of mixed phase We may visualise an ebb and flow of trophozoites to and from the peripheral circulation with a frequency determined by the number of broods With several broods the organisms seen in blood films would be of

THOMSON J G d M Lell S W (9) The cul va f one gen ra on f malarial par tes (*Plasm d um f l p rum*) in vitro, by Bas method *Ann tr p M d P* 6 449

mixed phase and blood films would remain positive throughout the cycle with a single brood, a rare occurrence in Malayan infections all the parasites would recede to the deep organs at about the same time and disappear from the peripheral blood stream for the last 12 18 hours of the cycle

Maurer (1902) seems to have been the first to relate the size of the ring forms of *P falciparum* in South East Asia with the pattern of the fever Working in Sumatra he showed that the small rings are in the majority when the temperature is rising and large rings are usual towards the end of the paroxysm and in the fever free intervals between paroxysms His observations are readily confirmed in Malayan infections when, from the dominance of one brood of parasites, the fever is tertian in type The infection of the patient whose temperature record is shown in figure 5 was dominated by though not restricted to a single large brood The relation between ring size and the course of the fever in this infection is clear, more often there are overlapping minor broods the temperature is irregular and the rings are of different sizes

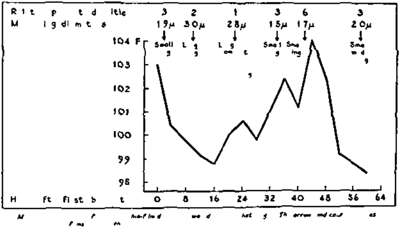


Fig 5—RING SIZE IN RELATION TO FEVER IN A SEVERE FALCIPARUM INFECTION OF MALAYAN ORIGIN (By courtesy of Lt Col M F H Kelleher British Military Hospital Kinrara Malaya)

Ring size and immunity—The six hour trophozoites may perhaps be accepted as the smallest and the thirty hour trophozoites as the largest normally seen in blood films these limits being determined by the stage at which the ring forms appear in and disappear from the peripheral circulation With an earlier appearance and disappearance the rings seen in blood films would of course be smaller This difference in the timing of the ebb and flow of parasites is a possible explanation for the differences in ring size which have been reported from different parts of the world particularly from Africa Wilson (1939) working in Tanganyika finds that the rings in non immune subjects tend to be monomorphic and very small Babies living in hyperendemic areas and Europeans infected for the first time show these minute rings By contrast the rings found in blood films from persons who are relatively immune are often large and pleomorphic Schwetz (1949) in the Belgian Congo reports similar differences in ring size in acute and chronic malaria, so do Shute (1951) and de Mello (1951) In the highlands of Kenya according to de Mello the chronic infections in the spring show relatively large rings

MAURER G 90 (f p s)
WILSON D B (939) I plications f malarial end micity I Ea t Africa *T ns R Soc t p M d Hyg 3 435*
SCHWETZ J (949) N t n d mi d acu malaria in Central African nati es *T ns R Soc p M d Hyg 4 4 3*
SHUT P G (95) *B m d J* 80
DE MELLO J P (1951) Surv y f mala m g th ind g ou population n th h ghland f K y w th ref t hypere d mi *E Af m d J 28* 465

while the acute infections of the late summer are associated with minute rings and frequent marginal forms

In Malaya this apparent relation between ring size and immunity is less clear Infections in which the rings remain in the peripheral circulation until they are large and pigmented are common enough in semi immune subjects from endemic areas but infections with large rings though often mild are sometimes severe and may even be fatal Indeed some of the largest falciparum trophozoites seen in blood films in Malaya—we refer to the general size of a whole brood and not to isolated parasites—have been associated with severe infection The trophozoites illustrated in figure 7 were drawn from films taken from an elderly Chinese shortly before death

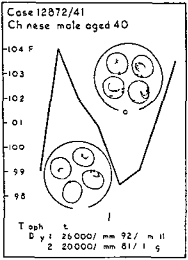


Fig 6—SMALL AND LARGE RINGS IN A MILD FALCIPARUM INFECTION OF MALAYAN ORIGIN

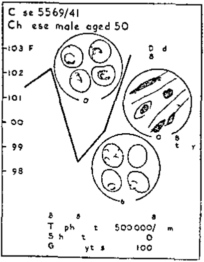


Fig 7—SMALL AND LARGE RINGS IN A FATAL FALCIPARUM INFECTION OF MALAYAN ORIGIN

Schizonts in the peripheral blood— The presence of forms of advanced development and in a state of segmentation in the circulatory blood is a certain indication of gravity Films from patients with serious infection may show parasites at all stages of schizogony the early dividing forms more often than the mature segmenters These infections are nearly always primary with a history of 5 to 10 days of untreated fever but they may sometimes be of many weeks duration Marchoux and Jolly (1935) report the case of a soldier with serious mental symptoms due to a falciparum infection contracted in North Africa ten weeks earlier Blood films taken at this late stage revealed an extremely heavy infection with one cell in every four infected and segmenting parasites at all stages

The segmenters in blood films are seldom numerous in relation to the number of ring forms they are always comparatively few for there is no general retention of segmenting organisms in the peripheral circulation Plate XXXIX drawn from a film taken just before death is a typical example in such cases it is possible that a few parasites pas through the complete schizogonic cycle in the peripheral blood or it may be that the capillaries in parts of the deep organs are over filled with segmenters and that a few leak back into the general circulation

M CHO X E and J lly A M D (1935) Cy l é l uf compl d P f lap non da l g irculan A I P ur 55 6 3

Table 2—THE PRESENCE OF SCHIZONTS IN THE PERIPHERAL BLOOD IN RELATION TO THE INTEN SITY OF INFECTION IN 3 450 ACUTE FALCIPARUM INFECTIONS OBSERVED IN MALAYA

	Parasite counts per c mm blood					
	Under 5 000	5 000—25 000	25 000—100 000	100 000—250,000	250 000—500 000	Over 500 000
Infections	753	1 498	974	147	46	32
With schizonts	10	21	60	40	20	20
Percentage	1	1	6	27	43	62

The relationship between parasite density and the presence of schizonts in films from the finger blood is shown in table 2 based on blood examinations before treatment in a series of acute falciparum infections naturally acquired in Malaya In these infections the presence of schizonts in blood films was usually an indication of clinical severity Their absence on the other hand did not necessarily imply a favourable prognosis , fatal infections in which segmenters were not found in blood films were by no means rare

Peripheral schizonts in mild infections—The early Italian workers regarded the presence of falciparum schizonts in blood films as a certain indication of gravity This too has been the experience of Shute who has never seen schizonts in mild infections induced for malario therapy in non immune subjects with falciparum stra ns from Rome and Sardinia

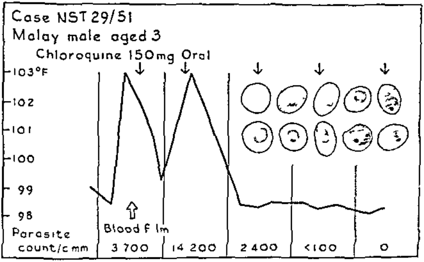


Fig 8—PERIPHERAL SCHIZOGONY IN A MILD FALCIPARUM INFECTION OF MALAYAN ORIGIN NINE SUCCESSIVE PARASITES FROM A BLOOD FILM TAKEN ON THE FIRST DAY OF TREATMENT

These findings however are not universal In Malaya the presence of schizonts in blood films from mild infections is by no means rare while in some parts of the world the phenomenon is apparently fairly common Perry and Bensted in the Leeward Islands de Zulueta in Columbia Raper *et al* in Kenya and Jolly in French Equatorial Africa all record the presence of falciparum

PERRY H M ND BENST D H J (933) *Pl sm d um f lcip rum* in th Le ward I l ds (Ant gua) S m unusual form not d in periph al blood film *T ns R S t p M d Hyg* 27 5
DE ZULUETA J (943) E quizog nia d l *Plasm d um falcip rum* en l circula n perif rica *Rev F M d B g t* 65 (Ab tr ct *T p D B ll* 41 259)
RAPER A B W LSON M RGARET E d W LS N D B (945) D viding f rms f *Pl m d um f l p rum* in t pe pheral bl od f Af cans *T ns R S trop M d Hyg* 38 91
JOLLY A M D (936) A pects typ qu du *Plasmod um f lcip m* he les nf t indigè es bas âg à Brazzavill (A. E. F). F éq ce d l luti n h goniq et gametog nique mplète dans le g periph riq *R Malariol* 15 29

schizonts in blood films from subjects with mild infections Shute has seen them in healthy carriers from the Entebbe and Kisumu areas of Uganda and Garnham finding schizogonic forms in blood films from 13 of 50 unselected falciparum infections states his view that these forms are less elusive in Kenya than elsewhere

How are these differences to be explained ? One suggestion is that they are related to host immunity Peripheral schizogony in mild falciparum infections has been reported mainly from countries where malaria is hyperendemic and immunity high it is apparently rare in non immune subjects it is uncommon in Malaya where the malaria though sometimes hyperen demic tends to have a low or moderate endemicity Another suggestion is that there are inherent differences in the parasites themselves that some geographical strains are less prone than others to complete schizogony in the internal capillaries The evidence on the whole supports the first suggestion but decisive proof must await an examination of the parasites under comparable conditions

Parasite density—The parasite concentration in falciparum infections may exceed 10^6 per c mm of peripheral blood vivax infections rarely attain a density of 10^5 per c mm quartan infections seldom exceed 10^4 per c mm The malignancy of *P falciparum* is related to this great proliferative activity infections with a peripheral density of parasites exceeding 10^6 or with 25 per cent of the red cells infected are usually fatal

Table 3—PARASITE DENSITY BEFORE TREATMENT IN 3 450 ACUTE FALCIPARUM INFECTIONS OBSERVED IN MALAYA

	Parasite counts per c mm blood						
	Under 5 000	5 000—25 000	25 000—100 000	100 000—250 000	250 000—500 000	Over 500 000	Total
Infections	753	1 498	974	147	46	32	3 450
Percentage	22	44	28	4	1	1	100

The density of parasites in the peripheral circulation of patients admitted with acute falciparum malaria to hospitals in Malaya may be assessed from table 3 based on parasite counts from finger blood made on the first day of treatment in a series of cases observed by the staff of the Malaria Research Division of this Institute A minor fallacy in the figures is that patients with counts less than 1 000 per c mm of blood were usually excluded

The heaviest Malayan infection of which we have an adequate record was revealed in a blood film sent to us in 1935 by Professor R B Hawes formerly of the College of Medicine Singapore The patient was admitted unconscious to the Tan Tock Seng Hospital Singapore recovered consciousness but died soon afterwards the parasite count was 1 860 000 per c mm of peripheral blood and 42 per cent of the red cells were infected Recently Major R Topping R A M C British Military Hospital Kinrara Malaya has shown us a film from a British soldier who died from an infection in which 52 per cent of the red cells contained parasites there were 76 parasites in every 100 red cells the parasite density was not known Springall (1943) and Macfie (1914) report fatal infections in which 44 and 47 per cent of the red corpuscles contained parasites while Chopra (1932) saw a fatal infection with 67 per cent of the red cells infected and a parasitaemia just before death at the almost incredible level of 2 800 000 per c mm

Why *P falciparum* should display this great proliferative activity is not known Is it because the segmenters produce more merozoites than other species that the curve of geometrical

GARNHAM P C C (1933) A n th subtertia malaria p te of K y *E Af m d J* *10* 68
SP GALL A N (1943) H avy d nsity f *P f l pa um* para tes n malari Report f case *Amer J trop M d* *23* 533
MACFIE J W S (1914) Not on f al d p on lly i tense infection with malaria (*P fal parum*) *Lanc* 1354
CH RA R N D G p B M and Se B (1932) A f tal ca f ere maligna tertian malari *Ind med Gaz* *67* 68

increase is steeper ? Have the merozoites stagnating in the capillaries of the deep organs a better chance of securing a hold on fresh host cells than those of other species swirled through the general circulation and exposed to the phagocytes ? Are the conditions of the internal capillaries peculiarly favourable for the proliferation of the organism ? Does binary fission come into the picture ? Does the fact that *P falciparum* invades the mature red cells rather than the immature cells selected by *P vivax*, give the young organism a wider choice ? Can it be that there is a malignant malaria parasite with a blood cycle of 24 or 36 hours as some of the early workers thought ? Was the organism adapted to *man later* than other species with a relative delay in its age long progress *towards* symbiotic association ? These are some of the theories from their number and variety we may presume that none is entirely acceptable

Multiple infection of host cells—The invasion of single red cells by more than one parasite occurs in all forms of malaria but in frequency and degree the phenomenon is most marked in falciparum infection So much so that a tendency to multiple invasion is a minor guide to species diagnosis Double, triple and quadruple infections are common In a fatal infection we have seen seven rings in a single cell , Springall (1943) has reported eight The cell containing seven parasites reproduced in plate IV was drawn from a film taken a few hours before death from a patient with a peripheral parasitaemia of over 1 000 000 per c mm and with 31 per cent of the red cells infected The degree of multiple invasion was related as one would expect to the intensity of infection , the proportion of cells invaded by more than one parasite closely agreed with the mathematical odds of random distribution

Total cells counted	Single infection of cell	Double infection	Triple infection	Quadruple infection	Quintuple infection
5 000	1 463	363	77	10	2

The parasites in multiple infected cells are nearly always ring forms of about the same age other combinations are possible though rare A ring may share a cell with a schizont or a gametocyte or two schizonts or two gametocytes may be found together (plates XI and XIV) We have never seen more than two segmenters in one cell—the three to a single cell illustrated by Cropper (1908) must be very rare—and it seems likely that most of the parasites in heavily infected cells either die from lack of nourishment, or else migrate to other cells

Intermittancy in parasite density—The ring forms of *P falciparum* normally disappear from the peripheral circulation when they are about 24 hours old but they are replaced by young rings from later overlapping broods and blood films are continuously positive There is a theoretical possibility however, that infections may be restricted to a single brood Parasites would then disappear from the circulation every other day To what extent may the dominance of a single brood confuse diagnosis ? May blood films free from parasites one day reveal a heavy infection the next? Garnham (1933) has shown that *P falciparum* in Kenya may disappear completely from the peripheral blood for several hours of the 48 hour cycle Dr T Wilson tells us that a few falciparum infections of great intensity almost restricted *to* a single brood occurred among British and Australian prisoners of war during the Japanese occupation the infections appeared in the blood films to be heavy very light and heavy on successive days (*see* also p 4–)

We have examined the daily parasite counts of some 3 450 acute falciparum infections with these possibilities in mind With light infections in patients with a tertian type of fever we have sometimes found that blood films were positive and negative on alternate days but we have no record of a single severe infection in which parasites vanished from blood films to appear in large numbers a day later and in very few serious infections was the ebb in parasite

C[illegible]ER J (1908) Ph[illegible] m[illegible] bu[illegible] d[illegible] ce of p[illegible] tes in th[illegible] periph[illegible] al circul[illegible] [illegible] fatal case of pern[illegible]ciou[illegible] malaria *T[illegible] R S[illegible] p M[illegible] d Hyg 1* 145
GARNHAM P C C (1933) A n[illegible] on th[illegible] [illegible]bt[illegible]rtian malaria paras[illegible]te [illegible] K[illegible]nya *E Af[illegible] m[illegible]d J* 10 68

density so marked as to fall below the microscopic threshold for a 100 field examination of a thin film With rare exceptions it seems *P falciparum* in Malaya has a multi brood development with no marked daily variation in the number of parasites in blood films The diagnostic significance of a fluctuating density of parasites is discussed in a later section (p 41)

Distribution in the body of the host—The ho t cells of the young organisms circulate freely and are distributed fairly evenly throughout the blood stream the older parasites and particularly the segmenters are normally restricted to the deep organs Marchiafava and Bignami state that the brain contains the largest number then come in order the lungs the spleen the bone marrow the liver and the intestines though the intestines may sometimes have the greatest number Seyfarth (1924) from an extensive study of fatal falciparum malaria in the Mediterranean area during the first World War found no particular localisation of parasites in about one third of the cases with the brunt of infection in the remainder falling on the bra n the heart and rarely the kidneys suprarenals and pancreas In a series of fatal falciparum infections studied by Woino Iassenetzky (1939) the distribution of para ites showed a wide variation with the greatest accumulations usually in the brain the adipose cellular tissues the mucosa of the alimentary can.l and the pancreas in pregnant women the main concentrations were in the inter villous spaces of the placenta and the capillaries of the breast Cerebral accumulations were restricted to subjects dying in coma For South East Asian infections recorded information on the distribution of parasites in the body is scanty among the 57 fatal falciparum infections observed by the Malaria Research Division of this Institute an overwhelming parasitaemia with no obvious localisation seemed to be th* usual cause of death The records are unfortunately incomplete from the losses of the Japanese occupation and from the difficulties of autopsy examinations in Muslims and no general conclusion on the distribution of the parasites is possible

The tendency to stagnate in vast numbers and in particular sites is one reason though not always the most important for the malignancy of *P falciparum* Accumulations in the capillaries of the brain may produce coma and other cerebral symptoms cardiac failure has been ascribed to a concentration of parasites in the minute vessels of the heart intestinal pulmonary renal and other symptoms have been similarly attributed to local accumulations of segmenting organisms These pernicious forms of malaria are well recognised though they receive perhaps a false emphasis in medical writings Most falciparum infections display no such dramatic localisation or clinical mimicry The history of the malarial fevers in Malaya leaves no doubt of their malignancy even so the average case seen in hospital has few symptoms other than those attributable to the fever Grave infections with anomalous symptoms do occur but they are not common more often malignancy seems to be an expression of the weight of unlocalised infection

Duration of schizogonic cycle—At the turn of the centurv supported by the immense authority of Marchiafava Bignami Celli Mannaberg and others the view was widely held that there are two kinds of malignant malaria parasite one with a 24 hour cycle of schizogonic development in the blood and one with a cycle of 48 hours This conclusion was not reached by direct observation of the schizogonic cycle for half of the cycle is hidden in the deep organs it came indirectly from studies of the temperature charts from blood examinations and from autopsy findings Another view was that of Thayer (1900) who accepting but one malignant parasite thought that the cycle varied from 24 to 48 hours The first study of the malaria parasites of Malaya was made by Wright (1901) when the existence of a malignant malaria

SEYFA TH C (1924) D malaria od *A ch S.hiff Tropenhyg* 8 289
WO NO-I SENETZ Y M S (1939) La dis rib i d p rasi da l gani me de l homm au cours d la malaria tropical *M d I aras* Moscow 8 3 (Ab ract *T op D Bull* 194 37 86)
W HT H 190 (ref p 8) Thay 900 (ref p 4)

parasite with a quotidian cycle was not seriously questioned From observations made in the General Hospital Kuala Lumpur whence comes much of the material for the present study Wright believed the duration of the cycle to be 48 hours in most infections but 24 hours in a substantial minority Craig working at about the same time in the Philippines thought that a malignant parasite with a 24 hour cycle could be recognised not only by the quotidian fever but from the general morphological characters of the organism He regarded it as a sub-species of *P falciparum* and gave it the name *P falciparum quotidianum* Little real support for the existence of a quotidian parasite has appeared since, though Marchiafava in 1931 still held that the malignant organism whether as a separate quotidian variety or from a life cycle of varying duration might sometimes segment every twenty four hours and Thomson and Woodcock (1922) make the defini e statement that the schizogonic cycle of the typical malignant tertian parasite of Southern Europe and the East lasts from 36 to 40 hours Covell (*1951*) *however, in* studies at Horton with falciparum strains from India Italy Sardinia Roumania West Africa, and the Belgian Congo found no evidence that the cycle was ever less than 48 hours

The main support for the theory that there is a quotidian form of *P fal*ˈ*iparum* comes from the quotidian type of fever and the small size of the presumed quotidian organism at all stages For the quotidian fever the prolonged and exact observations which are now pos..ible in the practice of malario therapy have brought another and more convincing explanation James and his colleagues (1932) and more recently Kitchen (1949) have shown that quotidian and tertian types of fever may occur in the cours~ of a single infection We may interpret this occurrence to mean (*a*) that in the same subject there are two kinds of *P falciparum* with cycles of 24 and 48 hours (*b*) that the organism has a variable cycle , or (*c*) that two generations of one organism with a 48 hour cycle may segment on alternate days The third explanation seems by far the most likely

No less equivocal is the morphological evidence for the existence of a quotidian organism The minute size of the rings may well be due to an earlier appearance in the peripheral blood or it may be that the small rings and schizonts are examples of the random variations which occur in the malaria organism as in most other forms of life

Direct observations on the growth of P falciparum are possible in artificial cultures and the general failure to demonstrate a 24 hour cycle *in vitro* affords additional though inconclusive evidence against a quotidian variety Field (1932) for example obtained cultures from 63 Malayan strains of *P falciparum—in the hospital where Wright thirty years earlier had reported* the presence of a quotidian organism—with no indication of such rapid growth as to suggest a 24 hour cycle and Shute with strains from Rome and Sardinia has found that most parasites *in cultures segment in about 36 hours the original rings being 6-8 hours old But evidence of* this kind is indecisive suggesting no more than the absence of a quotidian organism among the strains selected

Though the schizogonic cycle of *P falciparum* normally lasts 48 hours particular broods may reach maturity a few hours earlier or later leading to the changes known as *anticipation* or *r tard m m* in the timing of the temperature peaks associated with the aberran generations

CRA G C F (192) Th l ificati n and diff nt al di gn si of th a u o- utumnal malaria plasmodia *Amer J tr p M d I* 57

MARCHIAFAVA E 931 (f p 4)

THOMSON J D nd WOODCOCK H M Th para t of malar Chap 69 in *Th Prac of M d cine n h T p* Ed By m W and A hibald R G H nry F wd d *Hodd and S oughe n Lond* 192

COVELL G d NICOL W D (1951) Clini al chem th rape tic nd immun l gical tudies n d c d mal *Bri med Bull* 8 51 55

JAMES S P NICOL W D and SHUTE P G (1932) A tudy f indu d malignant t rtian mal ria *Pr R S M d* 25 1153

K TCHEN S F F lciparum malari Chapt 41 *M l riol gy* Ed M F B yd W B S u d rs Co London and Phil d lph 949

FIELD J W (193) *A R p Ins M d R F d Malay St* p 15

Duration of infection—Untreated or under treated falciparum infections tend to run an acute course with a cessation of activity within twelve months James *et al* (1932) in a series of 26 infections found the longest period of activity to be 3_ weeks Eyles and Young (1951) in some illuminating graphs show the course of the parasitaemia throughout the entire period of infection in 2_ subjects infected with a South Carolina strain The average duration was 222 days several infections lasted for a year one for 480 days Jeffery and Eyles (1954) in a small series of patients infected with a Panama strain found the mean duration of infection to be 279 days with a range of 114 503 days For South East Asian infections no reliable information is known to us

Survival of parasites after death of host—With the death of the host the parasites die too but not for some time While the body is warm they may survive for many hours according to Seyfarth (1924) they may even complete the schizogonic cycle though the merozoites never invade fresh corpuscles We have ourselves seen parasites in the brain capillaries with a lively movement in the pigment granules 18 hours after the death of the host For a few hours after death the young trophozoites in stained films from heart blood tend to retain their normal ring form with further delay many contract to appear as rather solid deeply stained bodies with central chromatin Chwatt (1948) found that the parasites in blood films taken from the right auricle 18 hours after death stained well and were easily recognised at 24 hours they were degenerate and at 36 hours unrecognisable The significance of these find ngs in the post mortem diagnosis of malaria is discussed in chapter VII

Survival of parasites in refrigerated blood—In sterile heparinised blood the parasites may survive for several days Infected blood packed in a thermos flask with chipped ice has been sent by air from Kuala Lumpur to Delhi Nairobi and London where the parasites—*P fal iparum* and *P knowlesi*—have been successfully transmitted to fresh hosts after 2 5 days Prolonged preservation is possible when infected blood is rapidly frozen in thin containers and maintained at a very low temperature

Cultivation of the parasites—Bass and Johns (1912) were the first to achieve the cultivation of malaria parasites through the complete schizogonic cycle Their method depends essentially on the addition of a small amount of dextrose to defibrinated infected blood The parasites grow well for the first generation but growth thereafter is capricious and few of the merozoites survive to enter fresh cells The technique is simple and the cultures are useful for observations on phagocytosis and on the morphology of falciparum segmenters It was thought that cultures might be useful too for the recognition of parasites in light infections but later experience tends to show that diagnosis from ordinary thick blood films is quicker easier and equally accurate and parasite cultures are seldom used to day as a diagnostic aid

The host red cells in these simple cultures have an extraordinary tendency to agglutinate into large masses when the parasites are about half grown the stage at which they normally recede from the peripheral blood to the deep organs (plate XLVII) This phenomenon first observed by Thomson and McLellan (1912) does not occur in cultures from other species and may perhaps be related to the peculiar behaviour of the organism at this stage in the human host.

EYLES D E d Y UNG M D (95) Th dur on of untreated inadequa ly tr ed *Plasmod um f l parum* inf ctions th h man hos *J na Mal Soc 10* 3 7

JEFFER G M and E L S D E (954) The dura in the h man hos f nf ctions w th P nama train of *Plasmodium f l p rum Amer J rop M d H g 3* 9

SEYF TH C (9 4) Mal riapara ten d Malariap gmen bef d an d Le ch *A h S hiff T penhyg 3* 35

CHW TT L J (948) D cuss on on p pe by Maegraith, B P th logical p ocess in malaria. *T an R Soc rop Med Hyg 41* 7 8

B SS, C. C. nd JOHNS, F M (9 2) The culuvation f malarial plasmodia (*Plasmod um vivax* and *Pl smodium falciparum*) vi *J p M d 16* 567

THOMSON J C ND M LELLAN S W (9) The cultiva on f on generat on f malarial paras t (*Plasmod um fal parum*) vi by Bas method. *A trop M d P as 6* 449

The simple Bass culture does not provide the physiological conditions necessary for the *in vitro* growth of the parasites much beyond the first generation Their complex nutritive needs are now better understood and elaborate media which permit growth over several generations have been devised, particularly by Geiman (1949) Valuable though these newer methods are for fundamental studies of the metabolism of the parasites they have no place in malaria diagnosis

GAMETOGONY

The phase in the life cycle of the malaria organism leading to the production of the mature sex cells or gametocytes is known as gametogony Beginning at some unknown point in the cycle pre determined maybe as far back as the sporozoite gametogony ends when the gametocytes die in the body of the host their destiny unfulfilled or pass to a new phase of development in the mosquito

Most of our knowledge of the gametocytes of *P falciparum* the familiar crescents comes to us from the early Italian workers though their biological role was known only when Ross, Grassi and others had traced the continuance of the life cycle in mosquitoes and McCallum had shown that they are sex cells destined to initiate this new phase of development A vast amount of detailed information has accummulated since but little of fundamental importance and we may perhaps take as our point of departure in this discussion the facts known to Marchiafava and Bignami in 1894

1 The crescentic bodies arise as ovoid or spindle shaped organisms which appear in the human host on the 7th or 8th day of fever These early forms do not reach the peripheral circulation except in severe infections Autopsy examinations may reveal them in large numbers in the bone marrow
2 The young ovoid or spindle shaped forms can be traced to the adult crescent forms which appear in the finger blood only after a further series of paroxysms
3 The adult crescentic bodies remain in the peripheral circulation for ten days or more after the cessation of fever, then they diminish in numbers and disappear
4 The crescentic bodies do not proceed to sporulation and they play no part in the clinical phenomena of infection

Origin—Gametocytes are generally thought to develop from merozoites thrown off in the course of the ordinary asexual multiplication of the organism As young trophozoites they are indistinguishable in appearance from the ordinary ring forms Parasites which can be identified as early gametocytes first appear in the bone marrow and spleen towards the end of the first week of fever when the organism has already passed through a schizogonic cycle in the liver and maybe half a dozen cycles in the blood Whence they arise whether as continuous offshoots from successive asexual cycles from the later cycles only or from some undisclosed source, is not known

Development—Beginning as ring forms in the spleen and bone marrow with no characteristic morphological features falciparum gametocytes can be identified with certainty only when they are nearly half grown They are then rounded or ovoid parasites with scattered pigment and undivided chromatin in host cells which are free from Maurer s spots Thereafter they lengthen become oat shaped then fusiform or crescentic and finally take on the sausage-shape familiar in blood films These stages are illustrated in plates XIII—XV

GEIMAN Q M I t m thod f tudy and culu at n of plasmod a Ch pt 9 n *Malariology* Ed M F Boyd W B S und rs Co London nd Philad lphia, 1949

The crescent wave—That crescents wax and wane in numbers in a fairly definite time relation with the attacks of fever was known to the early Italian workers but Thomson (1911) seems to have been the first to relate the crescent wave in the peripheral blood with the wave

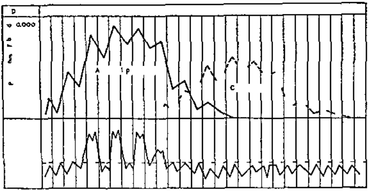


Fig 9—THE SUCCESSIVE WAVES OF TROPHOZOITES AND GAMETOCYTES IN FALCIPARUM MALARIA (after Thomson 1911)

of trophozoites some ten days earlier Beginning from seven to ten days after the onset of fever reaching a peak within a few days and slowly disappearing within two or three weeks after the fall of temperature the crescent wave seems to be a fairly stable feature in the life cycle of the organism

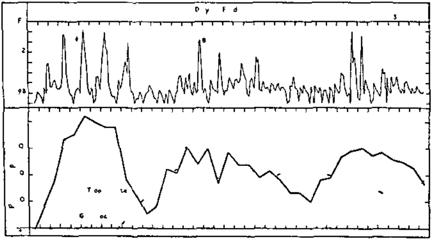


Fig 10—TROPHOZOITE AND GAMETOCYTE WAVES IN A PRIMARY FALCIPARUM INFECTION WITH PROLONGED INITIAL ACTIVITY (after Kitchen 1949)

THOMSO D (9) A res h in th p oduct on bf d d th f cres t in malign t t ru malaria, in tre ted and re d se by me ve m thod *A p M d P an* 5 57
KITCH S F Falcip rum mal Ch p er 4 in *M l riology* Ed. M F Boyd W B S nd rs Co Lo do d Philad lph 949

The appearance of crescents in the peripheral blood in the later stages of acute attacks, at a time when the defences of the host are coming into play, has led to the theory that immunity stimulates their production that the organism turns to a sexual mode of reproduction when conditions are unfavourable for the continuance of the asexual cycle Against this theory are the facts that crescent production is often high in primary attacks and in heavy infections when there is presumably little immunity, and low in the later stages of infection

The duration of the crescent wave after the end of a single falciparum attack is usually 2 3 weeks With an early relapse of infection a second wave may appear before the first is complete, and with a series of relapses at short intervals, or with smouldering infections, crescents may persist in the blood for several months The long persistence of crescents sometimes reported (Sinton *et al*, 60 days, Garnham, 128 days, Shute and Maryon, 62 days) suggests continued production in this manner

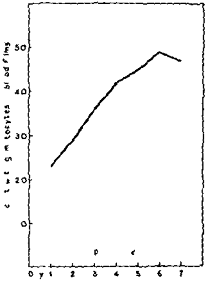


FIG 11 —DAILY GAMETOCYTE INCIDENCE DURING SCHIZONTOCIDAL TREATMENT IN 3,431 ACUTE FALCIPARUM INFECTIONS OBSERVED IN MALAYA

The effect on the crescent wave of schizontocidal drugs has often been debated During treatment crescents often increase in number This does not mean, as some have thought, that drugs stimulate production but merely that treatment coincides with the normal rise of the wave (fig 11) Drugs like quinine, mepacrine, and chloroquine given in full doses, tend on the contrary to lessen the number of crescents They modify the future course of the wave by arresting the production of crescents either at the source in the schizogonic cycle or when given early in the attack by arresting the early stages of crescent growth But there is less certainty that sub-clinical doses have this effect From their observations on subjects infected experimentally with a West African strain of *P falciparum*, Shute and Maryon (1951) came to the conclusion that small doses of antimalaria drugs do in fact stimulate the production of crescents

Numbers—Heavy falciparum infections in children living in hyperendemic areas often produce more gametocytes than the low grade chronic infections of adults (Christophers 1924 Wilson 1936), and it seems that the number of gametocytes produced in the individual person, or in the community is related inversely to the level of immunity This of course is the relation one would expect if the gametocytes arise from trophozoites as the production of trophozoites is greatly reduced by host immunity But there are wide variations Heavy crescent production sometimes appears after quite mild infections while heavy infections may produce relatively few crescents and it is possible that the host environment varies in its suitability as

SINTON J A BAILY J D AND CHAND D (1926) Studies in malaria with special reference to treatment IV The occurrence of sexual forms of *P falciparum* in the peripheral circulation *Ind Jour Med Res* 13 895

GARNHAM P C C (1931) Observations on *P falciparum* with special reference to the production of crescents *Kenya E Afr Med J* 8 2

SHUTE P G AND MARYON M (1951) A study of gametocytes in a West African strain of *Plasmodium falciparum* *Trans R Soc trop Med Hyg* 44 421

CHRISTOPHERS S R (1924) The mechanism of immunity against malaria in communities living under hyper-endemic conditions *Ind J med R* 12 273

WILSON D B (1936) Rural hyper-endemic malaria in Tanganyika Territory *Trans R Soc trop Med Hyg* 29 583

a medium for crescent growth Sinton *et al* (1926) suggest that the acidity of the tissues in the backwaters of the spleen and bone marrow may be important It is possible too that the organism itself may vary Jeffery (1951) for example found that an indigenous American strain lost the capacity to produce gametocytes after a series of transfers by blood passage

Table 4 HIGHEST GAMETOCYTE COUNT RECORDED DURING SCHIZONTOCIDAL TREATMENT IN 3,431 ACUTE FALCIPARUM INFECTIONS OBSERVED IN MALAYA

	Highe t gametocyte count per c mm blood				
	None	100	100-1 000	1 000 5 000	over 5 000*
Infections	1 -18	560	1 180	390	83
Percentage	35	16	34	11	2

I 4 inf cu th un d d 0 000 pe mm the highes d d wa 4 600

The incidence and numbers of gametocytes in our series is briefly analysed in figure 11 and table 4 Crescent counts were made daily for at least one week during treatment but it was seldom possible to follow the complete course of the wave

Proportion of males to females—The female gametocytes usually outnumber the males Various authorities have placed the proportion of females to males between 2 to 1 and 10 to 1 Green (19-9) working in Malaya in the hospital whence most of our patients were drawn found a wide variation with an excess of female gametocytes in most infections

Survival—The life span of crescents is difficult to determine for apparent long survival in the blood may simply mean a continued production If the crescents arise solely from the schizogonic cycle the elimination of the cycle would arrest production, and the duration of the crescent wave beyond this point would be a measure of their survival Normally they take about ten days to grow to maturity in the deep organs and often stay in the peripheral blood for a further 2 3 weeks after the arrest of schizogonic development This would give them a life of 3 4 weeks but prolonged survival has been recorded and the problem remains unsettled

BLOOD EXAMINATION AND PROGNOSIS

Opinion is strangely discordant on the value of blood examination as a guide to the outlook in—even to the presence of—the graver forms of falciparum malaria It is from the examination of the parasites state Marchiafava and Bignami (1894) that we can draw materials which enable us to form our prognosis of the dangerous fevers First and foremost we see that malignancy coincides with this exceptionally abundant quantity of parasites

JEFFER G M. (1951) Ob rv ons on gametocyteles trai f *Plasmodium falciparum. J nat M lar Soc 10* 337
GREEN R (19 9) Observation m factors influe cing th i fectivity of malarial gamet carri rs in Malay to *Anophel ma ul B ll N 3 f 19 9 Ins M d Re F d M lay S*
MARCHIAF AND BIGNAM 1894 (ref p 3)

Yet Lindsay (1943) fifty years later writing from a valley the malignancy of whose fevers had been noted even in the thirteenth century, takes the view that the microscope has little value in the diagnosis of pernicious malaria A negative blood slide he states has sent many to the grave Hunter (1945), from observations on some 3 000 cases of malaria in a forward treatment unit in one of the most malarious areas of the Assam Burma border protesting against an uncritical diagnosis of clinical malaria emphasises the ease with which parasites are found in serious cases and seeks to exorcise that *bogey man* of the medical officer' the cerebral case of malaria with persistently negative blood slides These two views are poles apart Within this extreme range there is a disposition to accept the evidence of blood films as reliable in general but sometimes misleading Khan (1945) for example in a series of some 20 000 cases of malaria treated on an Indian general hospital records 60 deaths from falciparum infection segmenting parasites were found in the brain of six cases though blood films during life were repeatedly negative and Wright (1941) believes that a sudden outset of coma in acute falciparum malaria usually caused by an embolism of parasites may occur when few or no parasites are found in blood films

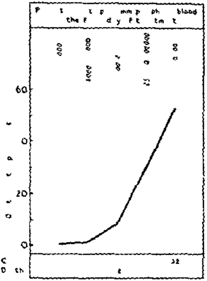


FIG 12 DEATH RATE ACCORDING TO PARASITE DENSITY IN ACUTE FALCIPARUM INFECTIONS OF MALAYAN ORIGIN (3 450 cases 57 deaths)

Greater confidence in the microscope has been expressed by others Lowe (1944) protesting against the belief that negative films from untreated cases of malaria are quite common states that he has not seen a case Ogborn *et al* (1944) support this view they saw no serious case of malaria in which the parasites were not numerous in blood films and in two only of a series of 378 falciparum infections were two successive films negative Field (1949) has expressed similar views

Prognosis and parasite density in untreated infection—With few exceptions malignancy in the falciparum infections of Malaya depends on the weight of infection A high density of parasites carries a high risk to life a low density carries a low risk With a peripheral concentration of parasites below 100 000 per c mm the death rate in our series was very low with a five to ten fold increase beyond this level it was more than 50 per cent (fig 12 table 5) We should perhaps emphasise that the patients were Asians usually from malaria endemic areas We have no data on the correlation between parasite density and clinical severity in the falciparum infections of immigrant Europeans

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LINDSAY D K (1943) Gu d ce not s n falciparum malaria *T at R S p M d Hyg 37* 63
HUNTER T A A F in hyper-end mic malar al area An initial nalysis f 2 9 3 case *Ind m d G 80* 247
KHAN N U (945) Ce b l malar *J R Army M d C rp 84* 263 (Ab tra t *T p D Bull* 1945 42 861)
WRIGHT F J (941) Ce b al malaria *East Afr a m d J 18* 226
LOWE J (944) S me comm mi co eption f malari *I d m d G 9* 207
OGBORN R S RA ER A H AND WRIGHT R J (944) Stud of malari n th Ea Afr Command *II A re d rais* f th di g os of malari f m oth f v rs in East African n ti es *East Afr n m d J 21* 101
FIELD 1949 (f p 43)

Prognosis and parasite density in treated infection—Prognosis from blood films becomes less reliable when the parasite load has been reduced by treatment Death may occur when the numbers have fallen to a level at which parasites would not be recognised in thin blood films and it is by no means rare for a patient to pass into coma when the number of parasites in the peripheral blood is falling rapidly Most fatal infections in our series maintained high counts until the time of death but a minority were then almost parasite free (table 5)

It is perhaps well to emphasise that treatment while reducing the prognostic value of blood examination does not necessarily obscure diagnosis Most infections can still be recognised microscopically while the fever lasts (figure 14)

Prognosis and the presence of schizonts in blood films—In Malayan infections with few exceptions falciparum schizonts appear in blood films only when the parasite count is high (table 2 p 28) In the infections of our series schizonts appeared in 171 and of these 27 were fatal At high levels of parasitaemia the presence of schizonts in the peripheral blood was highly unfavourable at low levels their presence did not necessarily imply a grave outlook as recovery was usual (fig 13) On the whole the evidence of this particular Malayan series suggests that the presence of falciparum schizonts in blood films while an undoubted danger signal had less prognostic significance than the peripheral density of parasites

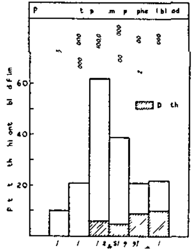


Fig 13 DISTRIBUTION OF DEATHS ACCORDING TO PARASITE DENSITY IN 175 PATIENTS WITH FALCIPARUM SCHIZONTS IN BLOOD FILMS

Table 5 TROPHOZOITE COUNTS IN 57 FATAL FALCIPARUM INFECTIONS OF MALAYAN ORIGIN

Case number		Daily trophozoite counts per c mm peripheral blood						
		Day 1	2	3	4	5	6	7
QST	355/39	1 336 000†						
—	362/39	1,200 000	773 000†					
NST	43/51	1 186 000	441 000	86 000†				
QST	338/39	1 000 000†						
—	190/39	939 000†						
PST	136/48	922 000†						
—	43/47	8_8 000†						
QST	408/37	770 000	530 000†					
TST	227/37	731 000	608 000†					
AMST	5/35	700 000	272 000†					
AST	73/35	650 000†						
QST	371/40	556 000	773 000†					
—	209/36	540 000	50 000	7 000†				
—	280/36	514 000	750 000	44 000	23 000†			
—	464/41	511 000†						

Table 5 (*continuation*) TROPHOZOITE COUNTS IN 57 FATAL FALCIPARUM INFECTIONS OF MALAYAN ORIGIN

Case number		Daily trophozoite counts per c mm peripheral blood: Day 1	2	3	4	5	6	7
QST	197/36	>500 000	220 000	28 000†				
AMST	35/35	>500 000†						
AST	44/35	450 000	369,000	228,000	48,000	2,800	300	0*
PST	129/48	4[illegible]6 000†						
TST	320/40	417 600	276 000	180,000†				
QST	396/37	369 000†						
—	392/37	350 000	370 000	50 000	4 000	1,200	800†	
	35/38	310 000†						
TST	335/40	306,000	120,000	54 000†				
QST	463/41	305 000†						
—	167/36	300 000	148 000†					
	330/39	290 000	108,000	300†				
NST	143/53	276 000	9 000†					
PST	10/47	274 000	364 000†					
—	182/49	258 000†						
—	284/50	251 000	70 000	7,400†				
QST	333/39	233 000	52 000†					
TST	311/40	229 000	160,000	66 000	31 000	1,500	<100†	0*
AST	45/35	2[illegible]8 000†						
PST	78/47	204 000	6,000	500†				
TST	212/37	185 000†						
QST	68/35	180 000†						
—	313/36	16[illegible] 000	349 000	371,000†				
—	423/37	148 000†						
TST	150/37	145,000	30,000†					
QST	424/40	124 000†						
—	430/40	110,000	7,400	<100†				
AST	26/35	108 000	659 000†					
QST	231/36	82 000	130,000	126 000	2,400	400	0†	
AST	15/47	80 000†						
CST	105/32	65 000	43 000†					
PST	19/47	65 000†						
—	34/47	61 000	3 000	100†				
QST	403/40	50 000†						
—	492/41	43 000†						
NST	56/52	41 000†						
AMST	2/35	35 000†						
CAMST	98/52	33 000†						
ACST	4/38	30 000†						
—	5/38	9 500†						
NST	120/52	3 500†						
QST	402/37	2 800	1 000	<100†				

This table excludes 9 fatal infections associated with other conditions contributing to or causing death—rupture of spleen 2, dysentery 2, pneumonia 1, marasmus 1, brain abscess 1, pulmonary oedema 1, anaemia (Hb 15 per cent) 1

These patients died on day 22

Falciparum malaria with few parasites in blood films— For a few hours before and during the early stage of the paroxysm very few parasites are to be found in the peripheral blood Welch (1897) in his original description of *P falciparum* states in these words a peculiarity of the organism which might be expected to confuse diagnosis The parasites of any

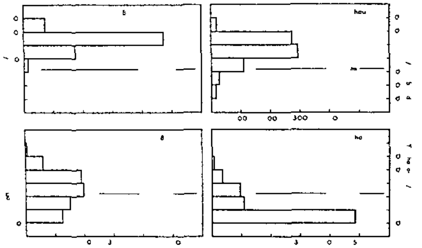


Fig 14—PARASITE DENSITY AND TREATMENT IN 736 QUININE TREATED ACUTE FALCIPARUM INFECTIONS IN RELATION TO THE MICROSCOPIC THRESHOLD FOR THIN BLOOD FILMS (after Field 1949)

particular brood disappear from the peripheral blood for the last 12 18 hours of the cycle and single-brood infections might not be revealed by blood examination during this period But Welch also recognised that segmentation usually takes place in successive groups of parasites and the implication of this further peculiarity is that the blood will remain continuously positive when groups of a sufficient size overlap To what extent we may ask does the first peculiarity lead to fallacies in diagnosis ? Does single brood infection ever lead to serious diagnostic or prognostic error ?

The infections of our series were all treated We are thus able to assess the daily fluctuations in parasite density only from counts made when the numbers were rapidly falling Even so in all but a few cases, the evidence against a significant fluctuation is fairly clear Blood films with rare exceptions were continuously positive until parasites finally disappeared from the peripheral circulation and we feel justified in assuming that Malayan strains of *P falciparum* develop with two or more overlapping broods The most marked daily variations in parasitaemia in the series are summarised in tables 6 and 7

F 1949 (ref p 43)

Table 6 DAILY FLUCTUATIONS IN PARASITE DENSITY IN MILD TREATED FALCIPARUM INFECTIONS

No		Trophozoite counts per c mm blood						
		Day 1	2	3	4	5	6	7
PST	304/50	56 000	6 300	91,000	3 800	24 000	5 400	17 000
—	194/49	40 000	3,900	6 700	400	19 000	100	31 000
—	188/49	18 000	75 000	600	49 000	2 400	0	—
—	199/49	17 000	9 600	0	3 500	0	400	0
—	424/52	8 200	9 500	0	1 700	0	1 300	0
BWST	38/52	6 500	400	3 600	300	1 900	300	2 600
PST	24/51	5 400	0	2,200	0	1,100	0	100
PST	440/52	4 900	0	300	0	200	0	—
BWST	54/52	3 800	400	12 600	200	3 400	200	300
PST	298/50	3 100	0	4 400	400	4 400	300	600
—	315/50	2 900	200	6 700	200	15 000	400	5 600
—	156/48	2 800	300	2 200	0	2 500	700	300

Table 7 DAILY FLUCTUATIONS IN PARASITE DENSITY IN TREATED FALCIPARUM INFECTIONS OF MODERATE OR MARKED SEVERITY

No		Trophozoite counts per c mm blood						
		Day 1	2	3	4	5	6	7
AST	56/35	562 000	4 800	54 000	3 000	100	0	0
TST	70/35	370 000	500	103 000	1 200	50	0	0
QST	401/40	200 000	15 000	100 000	11,000	700	0	0
AST	18/40	144 000	4 000	32 000	3 000	100	0	0
CST	56/33	144 000	2 800	26 000	500	0	0	0
AST	13/40	134 000	500	22 000	700	0	0	0
TST	263/40	124 000	100	16 500	6 000	0	0	0
—	309/40	104 000	600	13 600	100	0	0	0
PST	306/50	89 000	26 000	119 000	16 000	30 000	6 600	0

Some of the most marked daily variations in parasite density were found in a series of light infections treated by Dr J F B Edeson in the course of investigations on proguanil resistant malaria Blood films from several of these cases were negative on alternate days Here th lesser broods may have been suppressed by the proguanil an effect well known in knowlesi infections treated with sulphonamide , and the fluctuating parasitaemia may well have had an artificial cause (table 6)

This phenomenom of intermittancy in peripheral parasitaemia unusual though it seems to be in the falciparum infections of Malaya is a possible source of diagnostic error though apparently not a serious one Its occurrence emphasises the need for a daily examination of the blood for several days during fever when falciparum malaria is a possible cause and the wisdom of twice daily examinations in subjects who are seriously ill

A more difficult situation would arise were the schizogonic cycle of *P falciparum* confined to the deep organs, with little or no invasion of the peripheral blood Are there pernicious infections which, untreated never show more than a few rings in serial blood films ? This is a

problem of great clinical importance We have ourselves never seen a serious untreated infection in which the parasites could not be found with ease by repeated blood examinations over twenty-four hours but others have reported visceral infection with negative blood films and we cannot altogether dismiss the possibility that the organism may sometimes fail to reach the peripheral circulation We may recall the view of Golgi that the internal organs are the normal habitat of *P falciparum* in the human host and that the ring forms in the peripheral blood are there as it were by accident being washed into the circulation from their foci of development as the nucleated red corpuscles are conveyed from the bone marrow into the blood current in certain anaemias

The problem of malignant malaria with negative blood films has been discussed by Field (1949) who states his view that no diagnosis of pernicious malaria is valid in the persistent absence of parasites from blood films without the demonstration at autopsy of parasites in the deep organs Seldom is this proof given though a few workers have found parasites by brain autopsy when blood films during life had not revealed their presence What then should be done when pernicious malaria is thought to be a possibility and no peripheral parasites are found to support the suspicion ? Does clinical suspicion without microscopic proof justify the urgent attention which pernicious malaria demands ? None will dispute here the attitude of Nelson Jones (1944) that no patient should be allowed to die of untreated malaria because the disease appeared in an atypical form with negative blood slides and most will probably agree with his insistence on immediate treatment when there is adequate clinical evidence without waiting for positive results from blood slides Yet clinically sound though this attitude may be it should not mislead the physician into a belief that every suspected malaria patient who recovers when treated or dies without autopsy demonstration of the organism was necessarily suffering from malaria A diagnosis which may be clinically expedient is not necessarily valid evidence Confusion may arise from

- i The simulation of cerebral malaria by other diseases virulent pneumonia typhoid fever tuberculous meningitis head injury acute alcoholism may all produce symptoms at times resembling those of cerebral malaria (Wright 1941)
- ii The failure to recognise parasites in blood films the Romanowsky stains on which we rely almost wholely today for malaria diagnosis are subject to strange vagaries in the hands of those who are not using them so often as to understand their occasional capricious behaviour Most workers in malaria have seen examples of missed heavy infection attributable to defective stains poor technique or inexperience
- iii The effects of treatment

It is not easy to decide how far these sources of error have led to the view that serious even fatal malaria may occur without the parasites ever reaching the peripheral blood The possibility cannot be denied though in our view no supporting evidence is valid which does not meet the demands that

- i other possible causes of the symptoms have been excluded
- ii the parasites have not receded from the blood as a result of earlier treatment
- iii the blood has been examined at least twice during a 48 hour period
- iv the staining and microscopic techniques are reliable and
- v parasites are found at autopsy in vital organs

Incidental parasitaemia—The presence of malaria parasites in blood films from a sick person does not always mean that malaria is the cause of the symptoms In a community where

F EL J W (1949) Blood amina d progn cu f lcipa m malari *T R Soc op M d Hyg* 43 33
NELSO J NES A (1944) Mal g malar on th C ld Coas som linical no es *A p M d P* 38
W HT F J (194) Ce b l malari *E Af m d J* 18 6

the malaria is hyperendemic there are likely to be many symptomless parasite carriers, under these conditions any person, sick from whatever cause, may have parasites in the blood Coma from encephalitis or any other cause, associated with a few *falciparum* rings in the blood may be mistaken for cerebral malaria and a diagnosis of acute malaria in a patient with fever will usually seem reasonable when supported by a positive blood examination These are but two of the possibilities of error Diagnosis from blood examination must be tempered in these circumstances by clinical judgement Does the patient live in a malaria endemic area? Does the history of the patient suggest malaria and do the symptoms and physical signs support this diagnosis? Is there any other explanation for the clinical condition? Does the parasite density suggest acute infection? Yet whatever may be the cause of the symptoms the presence of malaria parasites in the blood of a sick person will always be a clear indication for specific malaria treatment at least for the first few days

Limit of parasitaemia consistent with life—The case mortality of falciparum malaria rises steeply as the concentration of parasites in the peripheral circulation increases beyond 250 000 per c mm less than half of our patients survived a peripheral parasite density exceeding 500 000 per c mm Parasite densities in 15 cases of our series surviving infections of this great intensity are summarised in table 8 The heaviest non fatal infection in the series was that of an *elderly Indian treated by Dr T Wilson for an infection in which the parasite density was* 1 272 000 per c mm of blood A curious feature of this infection was the absence of fever, after a rise of temperature to 100 4 F on the first day the patient remained afebrile throughout. Dr Wilson suggests that the extraordinarily high count may possibly have been related to haemo-concentration

Table 8 DAILY PARASITE COUNTS IN 15 PATIENTS SURVIVING INFECTIONS WITH A PERIPHERAL PARASITAEMIA EXCEEDING 500 000 PER C MM

No		Race, sex age	Trophozoites per c mm blood						
			Day 1	2	3	4	5	6	7
NST	190/54	Indian M 50	1 272 000	1,248 000	794 000	4 800	800	0	0
PST	224/49	Indian M 1	710 000	67 000	300	0	0	0	0
AMST	182/36	Indian M 30	662 000	414 000	170 000	6 000	0	0	0
NST	26/51	Indian M 12	640 000	95 000	700	100	0	0	0
QST	420/37	Chinese M 41	640 000	31 000	31 000	400	0	—	—
NST	5/51	Chinese M 53	635 000	71 000	9 000	0	0	0	—
QST	473/41	Chinese M -2	633 000	360 000	26 000	1,300	<100	0	—
QST	1/47	Indian M 27	630 000	190 000	2 600	100	0	0	0
NST	90/52	Malay F 4	608 000	34 000	400	0	0	0	0
NST	133/53	Chinese F 22	59- 000	500 000	16 000	1 100	0	0	0
NST	199/54	Malay M 27	575 000	43 000	3 400	0	0	0	0
AST	56/35	Indian M 40	562 000	5 000	54 000	3 200	<100	0	0
PST	24/47	Chinese M 7	550 000	297 000	7 600	<100	0	0	0
NST	141/53	Chinese M 11	525 000	85 000	8 000	0	0	0	0
QST	363/39	Sikh M 40	500 000	70 000	4,200	400	0	—	—

Two patients in the series survived infections in which 21 per cent of the red blood corpuscles were infected Dr A C S Hobson has shown us a film from a British soldier treated at the Military Hospital Kinrara Malaya who recovered from an infection in which 35 per cent of the red corpuscles contained parasites and Don and Meyer (1944) report a similar recovery with a 35 per cent infection rate of the red cells

DON C S D AND MEYER P F (1944) *An unusual cas f cerebral malaria. Brit med J* 49

Strahan (1948) supplements this information from his experience in Singapore where severe falciparum infections were common in British and Australian prisoners of war Using a continuous drip method of intravenous quinine treatment he saw recoveries in patients with a peripheral parasite density exceeding 1 000 000 per c mm an intensity of infection at which from pre war experience among Asians in Malaya a fatal outcome might well have seemed inevitable

Parasite counts in prognosis—The experience of the Malaria Research Division of this Institute points so clearly to the superiority of blood examination and particularly of parasite counts over clinical judgment in most cases of falciparum malaria not only for diagnosis but also as a guide to the chances of recovery that we commend to our clinical colleagues the adoption of routine counts in all serious cases Made before the issue is clouded by treatment they give information which can be got in no other way Few patients in our series died from uncomplicated infection when the peripheral parasite density did not exceed 100 000 per c mm at the start of treatment in marked contrast was the death rate of more than fifty per cent when the parasite load exceeded 500 000 per c mm The method of counting parasites is simple—a count of parasites per 1 000 red cells and a total red cell count give all the necessary data

VARIATION

With their immense numbers their high rate of increase often their geographical isolation the malaria parasites have rich opportunities for inheritable variation and adaptive change There is no lack of evidence that the parasites do indeed vary in their form and behaviour and we may hence presume that these opportunities are not entirely lost

The most clearly defined variations are those which modify the behaviour of the parasites in the human host The parasites may vary in their immunological characters in their susceptibility to drugs in the patterns of infection to which they give rise in the host and in the duration of events in their life cycle These biological differences involving the intimate constitution of the organisms are sometimes so constant and clear-cut that the existence of biological races or strains within at least two of the species infecting man is now generally accepted (James *et al* 1938 Ziemann 1938 Boyd 1940 Shute *et al* 1954)

Less well known are the morphological variations in the parasites The existence of morphologically distinct varieties is possible but the evidence is confused Parasites which are atypical in their form can be regarded as new varieties only when the abnormalities remain unchanged from generation to generation in a succession of fresh hosts and few of the many accounts of new forms satisfy this necessary condition

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STRAHAN J H (1948) Quinine by co tin ou in nous drip in th tre m t f cu fal parum malaria *T ns R Soc tr p M d Hyg* 41 669

JAME S P ND CI CA M (9 8 Species nd races f human malar paras te nd no mmunity *A t Conren Ter de M laria Morb P II* Ams dam, 69

Z EMANN H (938) Z m Probl m d Bildung vers hied R sen, V ri tät oder Spe es b i d Mala p as en. *ZN B k* 142 366

BOYD M F (940) On a ces of th malaria paras tes *Amer J trop Med* 20 69

SHUTE P G ND MA ON M. (954) A ontrib on th p blem f strains f human pla modi m. *R M lari I* 33 1

Biological variation—The biological differences in *P falciparum* which have been reported involve mainly the duration of the intrinsic incubation period the capacity to produce gametocytes the ability to develop in particular species of mosquito and the susceptibility to drugs Another biological variation recognised by every medical officer who has seen falciparum malaria in severe epidemic form, is that which determines virulence Perhaps as Micheletti (1929) suggests there are varieties of *P falciparum* with a special viscerotropism to which we may ascribe the frequency of pernicious infections in certain regions But virulence is an expression of a complex and obscure parasite host relationship and although mutations in the organism at a time of immense proliferative activity may be important other factors may well be involved

Morphological variation— The idea must be held fast states Mannaberg that the malaria parasites are living organisms which are not to be calculated in all their phases with mathematical accuracy but show variations within well marked limits Here we shall consider only the variations which seem to lie outside the normal limits accepting as normal the original description of Welch (1897)

The occurrence in particular regions of falciparum strains which do not conform in all respects with the classical Italian parasites has long been suspected, and variant forms have been described which have been regarded on morphological grounds as definite geographical varieties Marchoux (192-) for example states that the West African parasites differ from those prevalent in Italy and Macedonia that the parasites of Indo-China are intermediate between those of Europe and Africa and that it is even possible to distinguish the parasites of Greece from those of neighbouring Macedonia Garnham (1933) distinguishes the Kenya parasites from those of Europe Raffaele and Lega (1937) and Ziemann (1915) recognise differences between the European paras tes and those of Ethiopia and the Cameroons and we shall see later that the Malayan parasites do not accord in all respects with the classical descriptions of the European forms These distinctions however have not always been made by a direct comparison of the parasites under similar circumstances and factors other than variety or race may be involved New varieties created on morphological evidence must satisfy rather exacting criteria The distinctive morphology must remain unchanged when the parasites are passed through mosquitoes to fresh hosts it must be constant in different human hosts and unrelated to host immunity and it must be free from all suspicion of an artificial cause Few of the new forms of *P falciparum* described have been submitted to so rigorous an assessment

Morphological variation may involve the size and form of the trophozoites the amount of chromat n the size of mature schizonts the form and distribution of the pigment and the size and stippling of the host cell Table 9 summarises a few of the variations which have been reported from various parts of the world the drawings reproduced from original sources in plates IX XI XIX XX and XXI portray some of the abnormal appearances

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MICHELETTI E (19 9) Cit d by BRUMPT E 1949 (f p 18)
WELCH W H Malar D finiti n yn ym hi ry nd p l gy I *Sy tem f Pr t l M d* Loomis and Th mps n New Y k 1897
MARCHOUX E (922) Multipli té des r ce d na I ro f mes d par tes d pal d me *B ll Soc P t* 15 108
GARNHAM P C C (933) A no th b rtian malaria par *te f K y E Af m d J* 10 68
RAFFAELE G AND LEGA G (937) O rvaz on su d un eppo u p di *P falcip rum R M l l* 16 S r I 388
ZIEMANN H (1915) U b g n rug Malariaparas t nf rmen *Zbl B kt* Abt I Orig 76 384

Table 9 SOME VARIATIONS IN THE MORPHOLOGY OF *P falciparum* REPORTED FROM DIFFERENT PARTS OF THE WORLD

Origin	Abnormal features
Europe	The malignant quotidian parasite of Marchiafava and Bignami minute ring forms mature schizonts very small host cells shrunken (1)
—	The unpigmented quotidian parasite recognised by Mannaberg similar to (1) but schizogonic forms have no pigm nt gametocytes normally pigmented (2)
Philippines	Minute rings with relatively abundant chromatin small schizonts named by Craig *P falciparum quotidianum* (3)
West Africa	Rather like (1) small rings and schizonts no clumping of pigment in schizonts small plump gametocytes named by Ziemann *P perniciosum* also found in Formosa (4) and Brazil (25)
	Large amount of chromatin in young trophozoites (4)
	Little or no cytoplasm in trophozoites chromatin resembling anaplasmata similar findings in Brazil (5 6)
	Appearance of paired rings (7)
	Scattered pigm nt in sch zonts (8)
East Africa	Trophozoites remain n periph ral blood to a late stage host cells seldom shrunken and sometimes slightly enlarged mature schizonts fill or nearly fill cell (9) similar find ngs common in Malaysia (10 11) Schizonts sometimes in blood films from mild infections (9 12 13) similar findings in West Indies (14) and rarely Malaya (11)
East and Central Africa	Small rings in non immunes large rings in semi immunes related possibly to the time of appearance of the rings in and disappearance from the peripheral blood (15 17)
Tunisia	*Tenue* and *cau asi um* forms in severe infection (26)
Ethiopia	Rings larger than normal coarse chromatin and late appearance of pigment crescents shorter and plumper (18)
India	Marked distortion in cytoplasm and chromatin of trophozoites named *P tenue* by Stephens (19) similar distortion reported from East Africa (20) Sudan (21) and South America (22) and common in Malaya (11)
	Piroplasma like forms suspected by Wright to be related to blackwater fever (23) similar forms seen in black water cases from Rhodesia (24)

1 M hiaf va d B gn m 894 (ref p 3) M nnaberg 1894 (ref p 5) ; 3 Cra g 1909 (ref p 5) ; 4 Ziemann 9 5 (f p 5) 5 M ch and I gram 9 7 (ref p 65) 6 Bertar lli (19 6) Abstract *Trop Dis Bull* 11 35, 7 S phen 1924 (ref p 14) 8 J ll 1936 (f p 8) 9 Garnh m 1933 (ef p 29) M ure 90 (ref p 1) *Fv ns rudv* J lly 936 (ref p 8) 3 Raper *al* (945) (f p 8) 14 Perry and Bens ed 933 (ref p 8) 5 Wilson 1939 (ref p 6) 6 Schwetz, 1949 (f p 6) 7 de M llo, 195 (ref p 6) 8 Raffael and Lega, 937 (ref p 46 9 Stephens 9 4 (ref p 5) 2 Callanan, 9 6 (f p 54) Balfou and Wenyon 19 4 (ef p 5) 2 Thomson 9 8 (f p 54 23 Wrigh 9 (f p 1 8) ; 24 Thomson, 9 4 (ref p 19 5 Guimaraes nd Cort 9 9 Ab tract *Trop Dis Bull* 5 263 6 Vall in 9 9 (f p 49)

Adventitious variation—Among the commonest changes in the appearance of the malaria parasites or their host cells, in stained blood films are those produced artificially (*a*) during the preparation or staining of the films (*b*) by the optical conditions of examination and (*c*) by the effects of drugs and those which appear (*d*) in the course of severe infections

(*a*) The living parasites in the early stages of schizogony are amoeboid Their form changes continually as they thrust out or retract their pseudopodia or shift their hold on the cell When they are dried quickly in thin blood films they are likely to retain the variety of form which characterises the living state with whimsical amoeboid outlines and loose chromatin dried slowly they contract to their resting state and assume the familiar ring formation with firm outlines and compact chromatin The rate at which blood films are dried is hence a factor in the morphology of the trophozoites in fixed preparations The shape of mature falciparum gametocytes is similarly affected Quickly dried they are long and sausage shaped dried slowly they tend to be shorter and plumper dried excessively slowly as in thick blood films they may become oval or even round due to early maturation. We have sometimes thought too that a slight enlargement in cells infected with tenue forms of *P falciparum* may be adventitious due to rapid drying of the films, the tendency to enlargement being more marked at the thinner and quicker dried end of the spread Descriptions of morphological variations in *P falciparum* do not always indicate whether these factors are controlled There are days in the tropics when blood films dry in a few seconds there are humid days when they remain moist much longer Ioff (1930) drawing attention to the distorted parasites found in films which are rapidly dried, states that the rate of drying also affects the intensity of Romanowsky staining—quick drying lessens and slow drying deepens the staining of parasites and cell stippling—but we have been unable to confirm this difference

Distortion in *P falciparum* may be due also to mechanical stresses from spreading the blood as a thin film Band forms are probably produced in this way and a marked distortion of the parasites and host cells is not uncommon at the end of the spread

Anomalous appearances in the parasites or host cells may also be due to defective staining Maurer s spots are difficult to stain when the pH of the diluent falls below 7 0 and at about pH6 0 the cytoplasm stains so faintly as to be almost invisible Anaplasma like forms apparently without cytoplasm may well be produced in this way, and difficulty in demonstrating Maurer s spots is more likely to be related to variations in staining than to anomalies in the parasites themselves

(*b*) Differences in the colour of falciparum pigment have often been described Th pigment it is said may be black, dark brown greenish or yellowish Such statements have little meaning unless the optical conditions of examination are defined Pigment colour in stained blood films depends on the brightness of the light source on the focus and aperture of the microscope condenser on the colour transmission of the light filter used on the size and degree of fusion of the pigment granules and hence their transparency and on the extent to which the granules are veiled by the blue staining cytoplasm Under differing conditions of examination the same pigment may appear yellowish greenish or almost black and colour differences must hence be accepted with reserve unless the conditions are known to be comparable.

(*c*) The effects of drugs on the morphology of malaria parasites are well known Parasites damaged by quinine may show evidence of cytoplasmic irregularity and degeneration and Thomson (1914) explains the occasional small size of falciparum schizonts as a quinine effect (plate XI) The pigment granules of crescents affected by mepacrine or chloroquine often fuse into irregular masses or the pigment may disappear altogether Proguanil and

Ioff I (1930) Zur Morphologie d Parasiten d Malaria tropica (*Plasmodium immaculatum* Gra u F letti 891) *Zbl Bakt Abt I. Orig 116* 25

pyrimethamine may inhibit the division of chromatin These common abnormalities appear during treatment when their cause is fairly obvious occurring in patients whose history is unknown they sometimes lead to puzzling anomalies

(*d*) Marked distortions in *P falciparum* may appear in severe infections Under conditions which are perhaps related to a high aggressiveness in the organism or to a low resistance in the host tenue and band forms are not uncommon Villain (1929) drawing attention to this apparent relationship believes that the atypical forms which have been called *P tenue* and *P caucasicum* are not different species but simple variations of *P falciparum* associated with a heavy paras te density

A MALAYSIAN FORM OF P FALCIPARUM

Many of the variations listed in table 9 have been seen in Malaysian parasites The trophozoites may be excessively small or so large that two in a cell would inevitably touch The chromatin of the trophozoites may appear excessive and extreme variations in the form of chromatin or cytoplasm are not uncommon Apparent pairing of the ring forms has been observed schizonts are sometimes found in mild infections and the mature schizonts vary in size though they tend to fill or nearly fill the host cell Some of these variations lie within the normal limits some are incidental appearing for a brief spell in isolated cases some we are inclined to regard as adventitious We have compared the parasites prevalent around Kuala Lumpur today with those portrayed by Watson (1903) in the neighbouring town of Klang and by Maurer (190-) in Sumatra and we do not doubt that they are the same We have further compared the Malaysian parasites with the Rhodesian parasites so beautifully illustrated by Thomson (1924) with the North African parasites portrayed in the excellent colour plates of Marchoux and Jolly (1935) and with the East African parasites described by Garnham (1933) Here again the parasites appear to have essentially the same morphology with differences which are no greater than might be expected within a normal range of variation

Between these tropical or sub tropical forms and certain European forms however there are rather striking differences in the size of the infected cells and of the mature schizonts The red corpuscles infected with European *falciparum* are said to be usually shrunken and the segmenters illustrated by Marchiafava and Bignami (1894) occupy no more than about one third of the cell (plate XI) The host cells of the Malaysian and of certain African parasites on the other hand are seldom reduced in size they are normally unchanged and sometimes slightly enlarged while the segmenters fill or nearly fill the cell (plate X) These differences may seem to suggest that the European and Malaysian parasites are morphologically distinct varieties indeed that not only the Malaysian but other tropical forms too may differ in morphology from those occurring in Europe at the northern limit of the species We are reluctant however to accept this hypothesis James and his colleagues (1932) comparing the malignant parasites of Rome Sardinia India and West Africa under comparable conditions were unable to detect any noteworthy differences and Koch (1899) in a far flung enquiry embracing Italy East and West Africa Central America Malaysia and New Guinea came to the same conclusion Moreover the Italian parasites illustrated by Bastianelli and Bignami (1899) resemble those of

VILLAIN G (9 9 Observ ons ne d plasmod typ ques cours d paludi me gra *A h Ins Pas eu T m 18* 35 (Abstr ct *T p Dt Bull* 193 *27* 66)
WATSON 903 (ref p 4)
MAURE 90 (ref p 1)
TH MSO 1924 (ref p 9)
MARCHOUX J LLY 1935 (ref p 27)
GARNHAM 1933 (ref p 9)
MARCHIAF AND B NAM 1894 (ref p 3)
JAMES *et al* 932 (f p 3)
KOCH R (899) Cited by James *t al* 1932
BASTIANELLI G ND B GN M A (899) Sull truttur de p rass malarica e l pecı dei g metı dei parassıtı estı tunnalı *A Soc S d Mal I* 1 13

Malaysia, and if we accept a morphological distinction between the European and Malaysian forms we must, it seems, also accept, with Marchiafava and Bignami two morphological types in Europe For these differences we may postulate the occurrence of a number of fixed morphological varieties not clearly related to geographical distribution or we may assume with Mannaberg a range of fluid variation determined by external conditions it is this second explanation which, on the evidence available, we are inclined to accept

Table 10 THE CLASSICAL DESCRIPTIONS OF *Plasmodium falciparum* AND SOME APPARENT DEVIATIONS IN THE MALAYAN PARASITES

Fe tu	Mar hiaf nd B gn mi (894)		W l h (1897)	Mal yan
	Malignant t rti n	Malign nt qu tidian		
S e of s gment r	F *rms of fiss on may* b as large a half to two-thi ds f th s e of the d blood c rpu cl	*Forms of fi s o con* s d r bly small r (accord g to Man n b g on fif h t o thi d th f th d bl od pu scl)	*S gm nt do not g* lly d ne thi d t on half the e f th d blood corp l	*S gm ters ally* fill o arly fill th h t corp l
Cha g n h t c ll	Th malign t qu tid a d tertian al k cau th h t ll t hri k hr l up nd w t way — n th q t dian m ybe to third f th ir no mal ize		Th host ell fte be me hrunk cre d oth w d f rmed	S ld m h w th e ch g
S gme ers in blood films	E c pt in gr inf t th f ms of fi n ar n ry ly		S gment a tr m ly a n the p ph l c cul ti	V ry omm in e e f cti ; ual n mild f cti b t n t r
Ab of para tes f m blood film	Du ing th first f w h urs f th par *xysm t h e* m n t n of th bl od m y be n g ti	Th e rul no point n th cour of *th di whe we* g t n e g a t i ult	F f w hour bef nd d r g th ly st g *f th par xysm ty f w* p t f ny ki d ar to b f und in th cir l ting blood d t thi p d th y may b tir ly b nt	Bl od film likely to b pos co t *nuously*
Ab c of p gm t	Alw ys p gm t d	Sp l tion o ns c m pl t d b f th moeb h be com p gm ted	F m pp whi h p thr ugh th mo b d matu d gment g ph w th y f ma on f p gm nt w thin th p	Alw y p gment d

AN ETHIOPIAN VARIETY OF P FALCIPARUM

Raffaele and Lega (1937) have found differences in form and behaviour between the malignant parasites of Ethiopia and of the Roman Campagna which have led them to suggest the existence of an Ethiopian variety The distinctions lie mainly in the shape and size of the male crescents and in the size and pigmentation of the rings The male crescents of the Ethiopian form are said to be shorter and broader and the rings to be larger with coarser chromatin and a later appearance of pigment But as typical *P falciparum* occurs in Ethiopia and as the Ethiopian parasite may be found in Italy the authors use the expression Ethiopian variety *with no implication of a particular geographical distribution*

Later Giovannola (1938) from a direct comparison in neuro syphilitics of two strains from Rome and Ethiopia concluded that the Ethiopian strain differed in the following characters

1 Greater frequency of large rings
2 Pigment less and of lighter colour
3 Schizonts in the peripheral blood not restricted to grave infections
4 Male crescents shorter and broader from the appearance of young forms in the peripheral blood

Ma ch af va and B gnam 894 (ref p 3) Mannab g 893 (f p 5) W lc 897 (f p 46)
Raffaele and Lega 937 (ref p 46)
Giov nnola A (1938) C pp t p ali pp pe d *Pl m d m mm cul um Et t d d d ll I tut d S Pubbl R m* V l Part III pp 79 804

From these characters the author is inclined to identify the Ethiopian parasite with *P perniciosum* the West African parasite described by Ziemann He points out moreover that the characters of the Ethiopian form have been recorded in other malignant parasites of tropical origin and suggests that there are grounds for regarding the Ethiopian West African and similar parasites as a tropical variety with the name *P immaculatum* (=*falciparum*) var *perniciosum* (Ziemann 1915)

Mosna (1938) however from a similar comparison of two falciparum strains from Ethiopia and the Roman Campagna found no significant differences in morphology but there was a marked difference in the apparent virulence the attacks due to the Ethiopian parasite being shorter and the symptoms much less sever

From this somewhat conflicting evidence we may perhaps conclude that a clear picture of the morphology of Ethiopian and other tropical varieties of *P falciparum* i indeed there are distinct varieties in tropical countries has yet to emerge

DOUBTFUL SPECIES AND SUB SPECIES

The malignant parasites which have appeared to differ so much from *P falciparum* as to be placed in a separate species or sub species fall broadly into two groups—(*a*) parasites which show a great variety of form and abundance of chromatin in the trophozoites and (*b*) parasites which are smaller than *P falciparum* at all stages The first group is represented by the single species *P tenue* Stephens 1914 the second group includes the European malignant quotidian parasite of Marchiafava and Bignami the Philippines parasite *P falciparum quotidianum* Craig 1909 the West African parasite *P perniciosum* Ziemann 1915 and the parasite *P caucasicum* Marzinowsky 1916 from the shores of the Black Sea Not included in these groups is the anomalous parasite found by Emin (1914) in the Middle East and named *P camaranense* by Ziemann (1915) None of these parasites has been adequately studied in a succession of fresh hosts and under varying conditions of examination and none has received a general and lasting recognition as a valid species or sub species

Plasmodium tenue Stephens 1914—This organism described by Stephens (1914) from a single blood film taken in the Central Provinces of India is said to differ from *P falciparum* in the abundance of the nuclear chromatin and the extreme irregularity in the form of the young trophozoites (plate XIX) On this equivocal evidence Stephens created the new species *P tenue*

The same peculiarity had been recorded earlier by Balfour (1908) in the Sudan and was seen later by Balfour and Wenyon (1914) in films from West Africa and Persia (plate XIX) These authors seem to have regarded the organism as a casual variant of *P falciparum* in which for some reason not clearly understood the trophozoites were unusually active

Plasmodium falciparum quotidianum Craig 1909—Found by Craig (1909) in the Philippines and said to occur also in Cuba Panama and the Southern United States this parasite was regarded as a sub-species of *P falciparum* on the grounds of its small size and apparent segmentation every twenty four hours The same peculiarity had been recognised earlier by Marchiafava and Bignami (1894) in certain malignant parasites of Europe and recorded in Malaya by Wright (1901) Most authorities today would probably agree that the

MOSNA E (1938) Sulle malarie indotta d *Plasmodium immaculatum (f l parum)* ceppo etiop ceppo campag R mana. Ri P ar 73
STEPHENS J W W (1914) A new malaria parasite of man *Ann trop Med Paras* 8 9
BALFOUR A (1908) Cited by Balfour and Wenyon (1914)
BALFOUR, A. AND WENYON C M (1914) The so-called *Plasmodium tenue* (Stephens) *J trop Med (Hyg)* 17 353
EMIN A (1914) Une nouvelle d parasite de La ra *Bull Soc P* 1 7 385
CRAIG C F (1909) Cited by Craig C F and Faust E C *Clinical Parasitology* 5th Ed Henry Kimpton, London 1951
MARCHIAFAVA AND BIGNAMI 1894 (ref p 3)
WRIGHT 1901 (ref p 3)

size of the organism falls within the normal range of variation in *P falciparum* and that the quotidian cycle is unproven and on the whole improbable

Plasmodium perniciosum Ziemann 1915—This parasite, found by Ziemann in West Africa, and said to occur also in Formosa differs from *P falciparum* in several minor morphological features including the smaller size of the schizonts and gametocytes and the lesser production of pigment Later Ziemann (1938) re affirmed his belief in the separate identity of the parasite and was supported in this view by Giovannola (1938) and by Soares (1941) who ascribed some of the severe malaria infections occurring in Brazil to a malignant organism resembling *P perniciosum*

Plasmodium caucasicum Marzinowsky, 1916—Found in the blood of a child living on the shores of the Black Sea this parasite has small rings with abundant chromatin and small schizonts occupying about one third of the host cell (plate XX)

Plasmodium camaranense Emin (Ziemann, *1915*)—*Emin (1914) found this parasite* in pilgrims living on the island of Camaran in the Red Sea He believed it to be a small variety of *P vivax* and called it *P vivax* var *minuta* Ziemann (1915) thought that it resembled *P falciparum* more closely but was different enough to be considered as a separate species to which he gave the name *P camaranense* The morphological characters of the parasite are shown in plate XX redrawn from Emin s plate

ZIEMANN H (9 5) U b ge ruge M lari par t nf rm n *Zbl B k* Abt 1 *76* 384
ZIEMANN H (1938) Z m P blem d r Bildung rs hi d e Ra sen V ta n ode Sp b den Mal n p ra *Zbl B k* Abt 1 *142* 366
G OVANNOLA 1938 (f p 5)
SOARES H DE A (1941) M lar pernicios C d çoes m t rno de algun c d mal ia d do *Plasmod m perni um* (Z m 9 5) Ab tract *T p Di B ll* 1942 *39* 93
MARZINOWSKY E J (19 6) D diffé t s pèce du par t d l malaria *Ann Inst Past* *30* 43

CHAPTER IV

PLASMODIUM VIVAX (GRASSI AND FELETTI 1890)

NAMES

Plasmodium vivax the benign tertian parasite of English authors is the cause of the malaria fever variously known as spring tertian fever benign tertian malaria and vivax malaria Infections with *P vivax* are seldom dangerous to the host they are often associated with tertian fever and in parts of Europe they tend to appear in the spring to these clinical attributes we owe the expressions spring tertian and benign tertian Vivax infections in some parts of the world however occur at all seasons they are not always benign nor is the fever always tertian and the name vivax malaria is hence displacing the older names for the disease

HOST AND HABITAT

Parasites with a close resemblance to *P vivax* are found in monkeys *P cynomolgi* a common parasite of the Malayan long tailed macaque and *P schwetzi* of African chimpanzees are very similar in appearance to *P vivax* but they are not the same and so far as is known natural infection with *P vivax* occurs only in man Attempts to transmit *P vivax* to monkeys have usually been unsuccessful though transient infections have been produced artificially in chimpanzees

In the human host the only known sites of development are the parenchymal cells of the liver and the red blood corpuscles The sporozoites are thought to enter the liver soon after the host is bitten by infected mosquitoes and to pass through a primary schizogonic cycle in the parenchymal cells Nin days or so after the initial invasion some of the merozoites from this primary exo-erythrocytic cycle enter the blood stream where they attach themselves to red blood corpuscles to begin the familar asexual cycle others are thought to re-enter the liver cells and to begin a secondary liver cycle or series of cycles thereby forming a continuing exo-erythrocytic reservoir of infection which is thought to be an important cause of relapses

BEHAVIOUR

Plasmodium vivax is the main cause of chronic relapsing malaria The organism may survive in the human host for several years lying dormant for months at a time presumably in the fixed tissues The blood forms unlike those of *P falciparum* display little tendency to concentrate at particular sites during schizogony but remain in the peripheral blood stream throughout the cycle This may be one reason why pernicious forms of vivax malaria are very rare Schizogony tends to be synchronised the members of any particular brood reaching maturity at about the same time The rate of increase is high but the peak density rarely reaches a dangerous level Mainly for this reason uncompl cated vivax infections seldom cause death They are hence held to be benign though the victims may feel extremely ill

GEOGRAPHICAL RANGE

Though *P vivax* tolerates fairly high temperatures and thrives like *P falciparum* in the tropics and sub-tropics it is adapted to lower temperatures and penetrates more deeply into the temperate zones The limits of the species are imposed mainly by temperature and by the absence of suitable vectors In the tropics the development of the organism in mosquitoes may be completed in 8 days (Green 1934) at 15 C development takes about a month and under

GREEN R *Ann Rep for 1934 Ins M d Re F d Malay S*

natural conditions many mosquitoes do not survive to transmit infection , below 15 C the completion of the sporogonic cycle is unlikely For this reason the organism usually fades out beyond the 15 C (59 F) summer isotherm , indeed from the lack of vectors and for other reasons endemic vivax malaria usually ceases well short of this limit Adapted in the mosquito to a lower summer temperature than *P falciparum*, and often carried through the winter cold by a capacity for latency in the human host *P vivax* is the main cause of malaria in temperate climates The northern limits in Europe are the coastal areas of Holland and Germany in Asia the species extends to the Amur river in northern Manchuria in America small foci of infection occur as far north as New Jersey and Oregon on the Atlantic and Pacific seaboards The southernmost limits are apparently determined more by the absence of vectors than by temperature endemic infection ceases at about the 30th parallel n South Africa and South America and has almost disappeared from Australia—all far to the north of the January 15 C isotherm

Throughout the Malay Peninsula P vivax occurs in all malarious areas The relative prevalence of the three species of *Plasmodium* varies with the locality and season but foci are known where *P vivax has been consistently the dominant species over several years* The overall proportion of vivax infections in hospital patients is fairly constant at about one third of the total admissions for malaria (*see* fig 4 p 23 and table 20 p 68)

LATENCY

In the human host vivax infections may remain latent for many months clinically silent and unrevealed by blood examination They may be latent from the beginning declaring themselves only after a prolonged incubation period—a primary latency which may either be spontaneous or be induced by early suppressive therapy They may on the other hand enter into a latent phase after a normal primary attack or series of attacks adequately treated with schizontocidal drugs Whether early or delayed latency usually lasts from six to nine months suggesting that the phenomenon may be an adaptation to the seasonal cold of temperate climates designed to ensure the survival of the organism during the months when mosquito transmission is in abeyance

Prolonged latency in vivax infections has been reported mainly from Europe the United States, and Korea *where the transmission of malaria is seasonal* When *transmission goes on all* the year round as in some tropical countries latency is seldom well defined and may even be absent *as in the Chesson strain infections of New Guinea origin On these grounds some* workers recognise two types of vivax infection characteristic either of the temperate or of the tropical zone—*a non latent tropical type and a long latent type of sub tropical or temperate* climates The distinction is not without support, but the prolonged latency which has been reported from India and Madagascar *is seemingly anomalous* and Covell (*1954*) *suggests* that latency in varying degree may occur in vivax infections throughout the world excepting only the South West Pacific *For neither view is the evidence decisive Few vivax strains of undoubted* tropical origin have received a careful study among the few are strains from the South West Pacific When the behaviour *of strains from tropical Asia Africa* and America is better known, the degree of correlation between latency and geographical origin will perhaps appear in clearer focus

Covell G (954) A t on p r ct d cub u d la lap n vi ax mal n *J p Md (Hyg) 57 55*

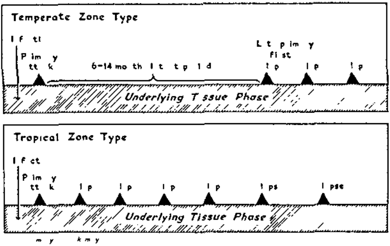


FIG 15—THE RELAPSE PATTERNS OF TEMPERATE ZONE AND TROPICAL ZONE VIVAX INFECTIONS (after Hankey *et al* 1953)

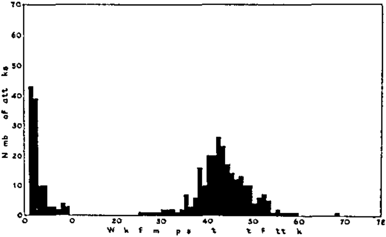


FIG 16—LATENCY IN THE ST ELIZABETH STRAIN OF *P vivax* INDIGENOUS IN THE UNITED STATES 368 ACUTE ATTACKS ARRANGED BY TIME OF ONSET AFTER EXPOSURE TO MOSQUITOES (after Coatney and Cooper 1948)

HA K Y D D J ES R J , CO TNEY G R AL O A S , COR W G G SON P I A DONOVAN W N (953 Nwe vi ax mal 1 N ral hi ry d ponse to chloroquine *Amer J trop M & H* g 958

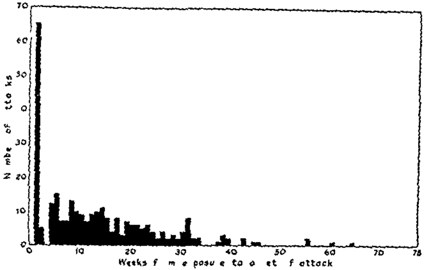


Fig 17—NON LATENCY IN THE CHESSON STRAIN OF *P vivax* OF NEW GUINEA ORIGIN 280 ACUTE ATTACKS ARRANGED BY TIME OF ONSET AFTER EXPOSURE TO MOSQUITOES (after Coatney and Cooper, 1948)

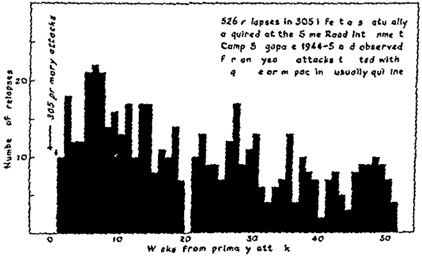


Fig 18—NON LATENCY IN THE VIVAX INFECTIONS ACQUIRED DURING AN EPIDEMIC IN SINGAPORE 526 RELAPSES ARRANGED BY TIME OF ONSET AFTER THE PRIMARY ATTACK (after Field Johnston and Carlile)

COATNEY G R AND COOPER W C (1948) Recrudescence and relapse in vivax malaria *Proc 4th Internat Congr Trop Med Mal* Washington 1948 p 6 9

FIELD J W JOHNSTON R S and CARLILE J P (Unpublished data)

During periods of prolonged latency in the human host *P vivax* is not found in blood films The mystery of its hidden retreat is still not fully solved James in 1931 thought that the clue might be found in tissue cells other than the red blood corpuscles Four years later Raffaele proved the accuracy of this assumption in the avian species *P elongatum* anticipating the recent discovery of Shortt and his colleagues of a cryptic stage of *P vivax* in the parenchymal cells of the liver Though there is no decisive proof there is good reason to believe that latency in vivax malaria is related to this hidden reservoir of infection and that a periodic leak of merozoites from this source into the circulation may be an important cause of relapses (Shortt *et al*, 1954)

INCUBATION AND PREPATENT PERIODS

The interval between the infecting mosquito bites and the onset of fever in vivax malaria is usually about a fortnight but may be prolonged for several months We may hence distinguish between normal and prolonged incubation periods while recognising that both may occur in different subjects infected with the same vivax strain Kitchen (1949) found the mean normal incubation period in a series of naturally induced infections to be 13 4 days with a range of 8 to 31 days James (1931) gives a mean of 13 6 days in 746 infections due to a Madagascan strain For Malayan strains no reliable data are known to us

Table 11—INCUBATION PERIODS IN PRIMARY VIVAX INFECTIONS (data from James 1931)

	Incubation period in days														
Infections	*−7*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*18+*	*Total*
Mosquito-induced	3	8	16	34	60	59	96	99	109	78	6	50	29	43	746
Blood induced	7	8	9	1[illegible]	14	5	1[illegible]	9	8	5	2	3	1	0	95

Prolonged incubation periods have been reported particularly with European American and Korean strains The incubation periods summarised in table 11 exclude a few infections occurring in subjects whose primary attacks did not begin until six months or more after the infecting mosquito bites Shute (1939) reports a mean delay of 28[illegible] days in the primary attacks of a selected group of patients infected with a Roumanian strain These long periods of incubation are apparently restricted to sporozoite-induced infections The period of incubation in blood induced infections is never prolonged and may in fact be shortened almost to vanishing point when the infecting dose of parasites is large

Malaria infections become patent when parasites can be detected in blood films Thick films give an earlier indication of infection than thin films and patency is hence determined as a rule by thick film methods The pre patent period in vivax malaria is usually shorter than the incubation period parasites being found in thick films before the host develops fever but a delay in the microscopic recognition of infection for the first two or three days of fever is not uncommon particularly in primary attacks occurring in subjects with no immunity In 428 infections naturally induced with nine vivax strains Kitchen (1949) found the mean prepatent period to be 12 2 days one day less than the mean incubation period The relation between the first day of fever of 100 F or over and the first detection of parasites was as follows

Parasites before fever	[illegible]75 cases
Parasites and fever on the same day	79 cases
Fever before parasites	74 cases

SHORTT H E, BRAY R S AND COOPER W (1954) Further notes on the tissue stages of *Plasmodium cynomolgi* *T ans R Soc trop Med Hyg* 48 13

KITCHEN S F Symptomatology general considerations Chap 4 in *Malariology* Ed M F Boyd W B Saunders Company London and Philadelphia, 9 9

JAMES, S P (1931) Some general results of a study of induced malaria in England *T ans R Soc trop Med Hyg* 24 477

SHUTE, P G (1939) Protracted incubation periods in indigenous cases of malaria in England *J trop Med (Hyg)* 4[illegible]

SCHIZOGONY IN THE RED BLOOD CORPUSCLES

Plasmodium vivax begins to invade the blood about nine days after sporozoite inoculation At first few in number, the parasites multiply rapidly and after a few schizogonic cycles in the blood corpuscles usually from one to three the host reacts with fever The parasites of any one generation tend to segment at about the same time—a synchronised development which contrasts with the irregular segmentation of *P falciparum*—and the paroxysms of fever tend to show a corresponding regularity Typically vivax infection is associated either with a single generation of parasites, segmenting every 48 hours, or with two generations segmenting on alternate days , in step with the maturing of these successive generations of parasites the fever is either tertian or quotidian in type A quotidian fever is usual in susceptible subjects at the beginning of infection a tertian fever suggests partial immunity This orderly development of one or two broods may fail however, during the first few days of fever in the primary infections of non immune subjects when parasites at all stages of schizogony may be found in a single film

Duration of cycle—The schizogonic blood cycle of *P vivax* lasts about 48 hours Young (1944) assessing the duration of the cycle in three American strains from the intervals between fever peaks found that the St Elizabeth strain reaches maturity in a little over 43 hours the McCoy strain in 48 hours and a strain from Baltimore in less than 42 hours Variations of several hours in the duration of the cycle may also occur in the course of infections with a single strain with *anticipation* or *retardation* in the timing of the paroxysms

Table 12—NUMBER OF MICROSCOPIC FIELDS EXAMINED FOR THE DETECTION OF THE FIRST PARASITE IN PRIMARY AND RELAPSING VIVAX INFECTIONS SIME ROAD INTERNMENT CAMP, SINGAPORE 1944 1945 *

	Primary attacks		Relapses	
First parasite detected in	Number	Percentage	Number	Percentage
First thick film field	12	15	297	53
2nd 10th field	16	19	151	·7
11th 50th field	25	30	82	14
51st 100th field	8	10	18	3
Not seen in 100 fields †	21	26	18	3
Total	82	100	566	100

* Blood e am tion on th first day of r c gn ed fe r † P tes n t s en on first d y but f und la r

Table 13—FIRST DAY ON WHICH PARASITES WERE DETECTED IN THICK BLOOD FILMS IN PRIMARY AND RELAPSING VIVAX INFECTIONS SIME ROAD INTERNMENT CAMP SINGAPORE 1944 1945

	Day of recognised fever				
Parasites first detected	1	2	3	4+	Total
Primary attacks	61	9	6	2	78
Percentage	78	11	8	3	100
Relapses	548	9	3	0	560
Percentage	98	2	0	0	100

Fever threshold—The immediate cause of the paroxysm in vivax malaria as in other forms of malaria is the rupture of a significant number of mature schizonts What this threshold concentration will be depends largely on the tolerance of the host Persons infected for the first

YOUNG M D (1944) Studies on the periodicity of induced *Plasmodium vivax* J nat Mal Soc 3 237

time tend to show a low tolerance so low indeed that symptoms may sometimes appear before parasites can be detected in blood films with partial immunity on the other hand a density of several thousand parasites per c mm of blood may fail to evoke a febrile response The diagnostic significance of this difference was brought home to us during the 1944 epidemic of vivax malaria at the Sime Road Internment Camp Singapore where most subjects were non immune and blood examination was possible on the first day of fever Parasites in relapsing infections were usually found in the first few microscopic fields examined while primary

Table 14—FEVER THRESHOLD IN 415 VIVAX ATTACKS OBSERVED BY BOYD (1940)

	Parasites per c mm blood on first day of fever								
	0	0–10	10–100	100–500	500–1 000	1 000–5 000	5 000–10 000	10 000+	Total
Primary attacks	52*	61	126	42	3	16	4	1	305
Relapses or recrudescences	—	—	2	14	14	61	17	2	110

* Par arli b t t n first day f f ver 15 p t t

infections were sometimes revealed only by a prolonged search of several thick films (tables 12 and 13) Most primary attacks according to the observations of Boyd (1944) are initiated by parasite densitie of 10 to 100 per c mm whereas a density more than ten times higher is usual in recrudescences and relapses (table 14)

Parasite density—Among the factors which determine the number of malaria parasites in the body of the host two seem to be of special importance—the innate reproductive capacity of the organism and the rate of destruction by the host defences At each 48 hour cycle *P vivax* multiplies about sixteen fold and for the first few cycles the number of parasites in the body mounts rapidly with successive cycles however the defences take an increasing toll until proliferation and destruction are in equilibrium at a peak density which seldom exceeds 30 000 parasites per c mm and rarely endangers the life of the host

Kitchen (1949) gives characteristic curves illustrating the rise and fall of parasitaemia in untreated vivax infections in fully susceptible subjects and in subjects who are partially immune from previous infection In the susceptible host the rise in density tends to be slow the maximum density sustained and the fall gradual Infections in partially immune subjects on the other hand are likely to show a fairly quick rise to a peak density and a quick fall The highest density he states rarely exceeds 50 000 seldom attains 40 000 and usually remains below 30 000 parasites per c mm The highest count observed was 96 000 per c mm a level which may be contrasted with the ten fold higher density sometimes attained in falciparum infections

Parasite counts observed in a series of vivax infections naturally acquired in Malaya are crudely summarised in table 15 The counts were made on the first day of treatment the patients were Asians with varying tolerance the infections were at varying stages and the attacks of varying duration when treatment began

Boyd M F (1944 O th par te density prevailing t certain period in vivax malaria infection *J na Mala Soc* 3 59
Kitchen S F (1949) V vax malaria Chap 43 in *Malariology* Ed M F Boyd W B Saunders Co. London nd Philadelphia.

Table 15—PARASITE DENSITY IN 1 191 ACUTE VIVAX INFECTIONS NATURALLY ACQUIRED IN MALAYA

	Asexual parasites per c mm blood				
	Under 5 000	5 000 25 000	25 000 100 000	Over 100 000	Total
Infections	394	660	136	1	1,191
Percentage	33	55	12	—	100

The highest count was more than 100,000 per c mm of blood The subject was an elderly Chinese, he was not seriously ill and his temperature at no time exceeded 100 F during an *uneventful* recovery on quinine treatment There were no deaths in this series of more than one thousand cases, and it seems that the death rate in Malaya from acute uncomplicated vivax infection is low

Selective invasion of reticulocytes—The immature red cell is apparently more susceptible to attack by the malaria parasite than is the mature cell This phenomenon first reported by Craik (1920) and studied by Eaton (1934) Kitchen (1937) and Hegner (1938) though apparently of little account in falciparum and quartan malaria may perhaps be significant in vivax malaria *Plasmodium vivax* is believed to have a strong preference for reticulocytes which is not shared by other species To this preference suggests Hegner may be related the low malignancy of vivax infections

Distribution in host—The complete schizogonic development of *P vivax*, from early ring forms to mature segmenters can be seen in serial blood films Unlike *P falciparum* which normally segments only in the capillaries of the internal organs, *P vivax* is distributed throughout the body at all stages of the schizogonic cycle But the distribution is not uniform Mature segmenters in blood films are nearly always fewer than the trophozoites from which they arise and it seems that an appreciable proportion circulating maybe less freely on account of their large size complete their segmentation in the internal capillaries Marchiafava and Bignami state that segmenters may be found in considerable numbers in the spleen and in fatal infections according to Brumpt (1949), they may be abundant in the various organs

Mixed infections—When two species of malaria parasites are found in blood films one species usually outnumbers the other Boyd and his colleagues from their observations on mixed infections induced by the simultaneous inoculation of *P vivax* and *P falciparum* suggest that the explanation may lie in a competitive antagonism between the two species When patients are infected with the two species at the same time *P falciparum* tends at first to suppress the multiplication of *P vivax* and to dominate the clinical and parasitological picture *P vivax* becoming active only at a later stage when the numbers of *P falciparum* are declining When however *P vivax* has the initial advantage over *P falciparum*, given by earlier inoculation, the two species may apparently maintain themselves in the peripheral blood without mutual interference (Shute 1951) The studies of Mayne and Young (1938) suggest that a similar suppression of one species may occur in simultaneously acquired vivax and quartan infections with *P vivax* as the usual dominant species We may perhaps infer from these experimental findings that mixed infections are commoner than single blood examinations would seem to indicate

CRAIK R (19) The erythrocytes in malaria *Lancet* 1110
EATON P (1934) Susceptibility f d ll t malari A p liminary note *Amer J t p M d* 14 431
KITCHEN S F (1937) The nf ction f ticulocyt by *Plasm d m* *Amer J trop M d* 18 347
HEGNER R (938) Rel u e f qu ncy of ring-stage pla modia in eticulocytes and mature erythrocytes in man and m nkey *Amer J Hyg* 27 690
BRUMPT, 949 (*f p 18*)
BOYD M F AND KITCHEN S F (937) S multaneous inoculation with *Pl smod m vivax* and *Plasmod um f lciparum* *Amer J t p Med* 17 855
SHUTE P G (195) M qu t inf ction in art ficially induced malaria *Bri m d Bull*, 8 56
MAYNE, B AND YOUNG M D (1938) Antagonism between species of malaria paras tes in induced mixed infections *Publ Hlth R p Wash* 53 1289

Relapses—The tendency to recur until they finally burn themselves out is a characteristic of vivax infections throughout the world The infections may be suppressed indefinitely with schizontocidal drugs and they may be cured by rather exacting courses of treatment with drugs of the 8 amino quinoline series but a recurring clinical activity is likely when the parasites are neither suppressed nor eradicated by treatment The immediate cause of the clinical relapse is the renewed activity of the schizogonic forms in the blood—a reactivation which may be due either to a fresh invasion of the blood from a reservoir in the fixed tissues or to an increase in the parasites already in the blood stream Relapses after many months of clinical quiescence are probably due to a re invasion of the blood from the fixed tissues relapses which occur repeatedly at short intervals are more likely on the other hand to be related to a renewed proliferation of parasites already in the blood stream

The number spacing and duration of relapses constituting what may be called the relapse pattern vary widely in different subjects The infections due to latent and to non latent strains of *P vivax* for example differ in their natural course and the relapse pattern is greatly modified by treatment

The relapses seen in clinical practice are likely to be mainly those which occur in the course of infections which have been treated only with schizontocidal drugs given during acute attacks So treated vivax infections may sometimes recur at intervals of a month or so for several years The observations of Coggeshall (1945) show that nine tenths of a large series of infections contracted in the South West Pacific relapsed at least four times more than half of them relapsed at least ten times and a small proportion thirty times or more—an extreme example of a relapse tendency for which South West Pacific infections seem to be notorious The vivax infections contracted in the Sime Road Internment Camp Singapore early in 1944 displayed

Table 16—THE FREQUENCY OF RELAPSES IN 305 VIVAX INFECTIONS CONTRACTED DURING AN EPIDEMIC AT THE SIME ROAD INTERNMENT CAMP SINGAPORE 1944 1945†

	Number of relapses during twelve months observation										
	0	1	2	3	4	5	6	7	8	9	Total
Cases	117	41	41	34	26	26	11	5	2	2	305

a similar if less marked tendency to recur The attacks were promptly treated with quinine or mepacrine a few were suppressed for limited periods with one or other of these drugs The frequency of relapses from the start of the outbreak to the end of the period of observation in August 1945 is summarised in table 16

Duration of Infection—Whether acquired in the tropical or in the temperate zone vivax infections tend to run a prolonged course if they are not treated with curative drugs Many infections display a sporadic activity for a year some for two years and a few for three years or more Hankey *et al* (1953) state that naturally acquired Korean infections end spontaneously within two years a period which may perhaps reflect the life span of the tissue stage of the Korean organism Hill and Amatuzio (1949) however report a duration of four years in a small

The term relapse used here in a loose sense to denote any recurrence of clinical activity after complete remission of symptoms regardless of the phase of infection. The terms recrudescence *relapse* and recurrence recognise the differing significance of renewed activity at different stages of infection James (1931) defines recrudescence as return of fever and parasites within eight weeks of recovery from the primary attack; return of fever and parasites later than eight weeks and earlier than twenty four weeks after recovery from the primary attack he regards as relapse; and still later return of fever and parasites as recurrence These definitions are arbitrary and have not been generally accepted. Coatney and Cooper (1948) reserve the term recrudescence for the type of relapse which can best be explained by the survival of erythrocytic parasites while recognising that it may not always be possible to distinguish recrudescence from true relapse arising from fresh invasion of the blood In this short account these refinements of distinction would serve no useful purpose (James, S P 1931, *Trans R Soc trop Med Hyg* 24 477 Coatney G R and Cooper W C, *Proceedings Fourth Internat Congr Trop Med Mal* Washington 1948 p 629)

† [illegible] published observations of Field, Johnston and Cahill

COGGESHALL, L T (1945) Cited by Kitchen 1949 (ref p 59)
HANKEY [illegible] (1953) (ref p 5[illegible])
HILL, E A and AMATUZIO D S (1949) Southwest Pacific vivax malaria. Clinical features and observations concerning duration of clinical activity *Amer J trop Med* 29 203

Table 17—DURATION OF VIVAX INFECTIONS OF SOUTH WEST PACIFIC ORIGIN (data from Hill and Amatuzio 1949)

Duration of infection in years	—1	—2	—3	—4	Total
Infections	38	82	28	7	155
Percentage	25	53	18	4	100

proportion of a series of infections acquired in the South West Pacific region (table 17) The infections were suppressed at first by drugs suppression ceased when the subjects returned to the United States, relapses were treated with schizontocidal drugs

GAMETOGONY

The gametocytes of *P vivax* like those of *P falciparum*, develop in the deep organs particularly in the spleen where they may be found at all stages of development The young forms do not regularly enter the peripheral circulation though they are not uncommon in blood films taken at an early stage in recurrent attacks, particularly according to Shute on the first day of long term relapses The adult gametocytes usually enter the circulation in primary attacks several days after the onset of symptoms, James (1932) working with a Madagascan strain, sometimes found them as early as the third day and exceptionally as late as the sixteenth. In relapses they may appear earlier even on the first day As infection progresses they increase in number but, unlike the gametocytes of *P falciparum* they usually disappear during

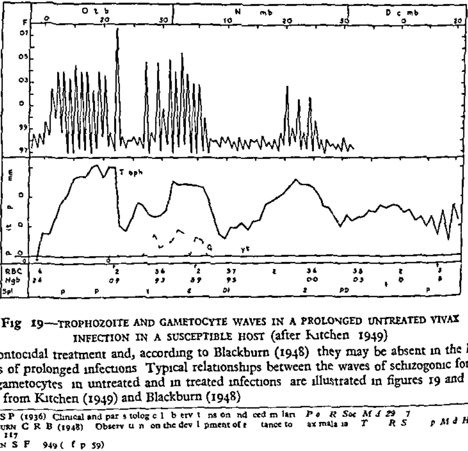


Fig 19—TROPHOZOITE AND GAMETOCYTE WAVES IN A PROLONGED UNTREATED VIVAX INFECTION IN A SUSCEPTIBLE HOST (after Kitchen 1949)

chizontocidal treatment and, according to Blackburn (1948) they may be absent in the late stages of prolonged infections Typical relationships between the waves of schizogonic forms and gametocytes in untreated and in treated infections are illustrated in figures 19 and 20 taken from Kitchen (1949) and Blackburn (1948)

J m s S P (1936) Clinical and par s tolog c l b erv t ns on nd ced m lari *P o R Soc M d* 29 7
BLACKBURN C R B (1948) Observ u n on the dev l pment of r tance to ax mala ia *T R S p M d Hyg* 4 117
KITCHEN S F 949 (f p 59)

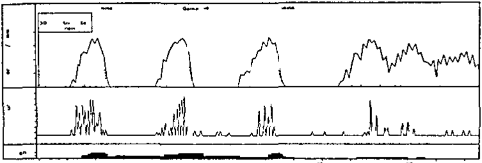


Fig 20—TROPHOZOITE AND GAMETOCYTE WAVES IN A SPOROZOITE INDUCED VIVAX INFECTION OF SOUTH WEST PACIFIC ORIGIN EARLY ATTACKS TREATED WITH QUININE (data from Blackburn 1948)

The number of gametocytes varies in different subjects though counts of several hundreds per c mm of blood are usual in acute infections Variations in gametocyte density may occur too in different vivax strains examples having been reported of strains which failed to produce

Table 18—HIGHEST GAMETOCYTE COUNT RECORDED DURING SCHIZONTOCIDAL TREATMENT IN 1 191 ACUTE VIVAX INFECTIONS NATURALLY ACQUIRED IN MALAYA

	H ghest g metocyt cou t pe mm blood					
	N ne	U d 00	00-000	000-5 000	Ov 5,000	Total
Infections	88	116	813	157	17	1 191
Percentage	7	10	69	13	1	100

I 4 infecu th ou t xceeded 000 the highes d d w 7 000 pee mm.

gametocytes after repeated blood passage to fresh hosts The daily gametocyte incidence during treatment in a series of acute vivax infections observed in Malaya and the highest number recorded are summarised in table 18 and figure 21

Unlike the gametocytes of *P falciparum* vivax gametocytes are destroyed by schizontocidal drugs they either disappear from blood films during the first few days of treatment or else they persist only as degenerate forms with little remaining but pigment and a few tattered remnants of cytoplasm The sharp contrast between the rising gametocyte incidence during schizontocidal treatment in falciparum infection and the falling incidence in vivax infection will be clear from a comparison between figures 11 and 21

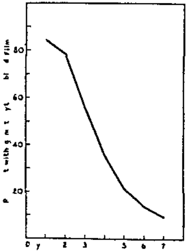


Fig 21—GAMETOCYTE INCIDENCE IN 1 191 ACUTE VIVAX INFECTIONS NATURALLY ACQUIRED IN MALAYA ILLUSTRATING THE MARKED FALL DURING SCHIZONTOCIDAL TREATMENT

VARIATION

Sharply defined differences have been reported in the behaviour of differing strains of *P vivax* and there is clear evidence for the existence of biological races and strains The differences involve mainly the duration of the incubation and prepatent periods the duration of the schizogonic cycle the capacity for latency, the frequency and timing of relapses the response to drugs, and the ability to complete sporogony in particular mosquitoes

Forms of *P vivax* which are abnormal in appearance are fairly common Atypical forms occur mainly when there has been some change in the life cycle or in the conditions of life, of the parasite they have been reported particularly in strains passed repeatedly from host to host by blood inoculation, and in chronic infections with a presumedly unfavourable host environment They involve mainly the size and shape of the infected cells the shape and texture of the trophozoites, the size of the schizonts and the number of merozoites The most common abnormal forms are perhaps the small schizonts with few merozoites which occur not only in blood transmitted and chronic infections but also, according to the observations of Young and Eyles (1949) as an occasional aberration in normal acute infections at the time of maximum parasite density

Marchoux (192-) believed that there might be geographical varieties of *P vivax* with characteristic morphological differences that it is possible for example to distinguish between the *P vivax* of France, North Africa and the Amazon For this view however, there seems to be little real support Few of the aberrations reported suggest permanent inheritable change most have either been found in a few subjects only often in a single film or else they have been fleeting changes occurring under abnormal conditions Some of the deviations from the normal appearances are summarised in table 19

Table 19—SOME VARIATIONS IN THE MORPHOLOGY OF *P vivax* REPORTED FROM DIFFERENT PARTS OF THE WORLD

Origin	Abnormal features
Malaysia	Marked multiple infection of host cells (1) (2) small schizonts with few merozoites (2) (3), ovale like morphology (4), bizarre forms (5)
India	Multiple infection of cells, schizonts with few merozoites, in a congenital infection (6), oval and fimbriated host cells, extra cellular appearance of parasites (7)
Paki tan	Gross enlargement of host cells with frequent multiple infection band forms common occasional absence of pigment (8)
Ceylon	Band forms during epidemic (9)

YOUNG M D AND EYLES D E (949) Paras tes re embling *Pl mod um ov l* in st ain *f Pl m d um* *J nat Malar S 8 2 9*

MARCHOUX 192 (f p 10)

1 RODENHUIS Y (19 1) *Gene k T jd ch Ned I d 61* 1 3
2 FIELD J W (942) *P as t logy 34* 82
3 — AND NIVEN J C (1939) *I d m d G 74* 509
4 — (1941) *B ll Ins M d R s F d M l v St N 4 f 1941*
5 NEEB H M (191) *T ns S c nd Congr F Eas A t op M d* Ho g Kong 1912 p 306
6 DAS GUP B M (1939) *Ind m d G 74* 273
7 SINGH JASWANT AND NAIR C P (950) *I d J M l* 4 193
8 MOHI UD-DIN A AND RAHIM A (1951) *P k t J H alth 1* 9
9 SAMARASINGHE D S PIYASENA A K. AND RUS OMJEE K. J (1944) *J M la Inst Ind* 5 397

Table 19—(*continuation*)

Origin	Abnormal features
New Guinea	Occasional marked multiple infection of host cells and ovale like forms in Chesson and Pait strains (10)
Sudan	Marked multiple infection of cells (11)
Middle East	Multiple infection and absence of enlargement in host cells containing Schuffner s dots small schizonts with fewer merozoites—the so-called *P vivax* var *minuta* Emin 1914 (12)
Israel	Absence of pigment in schizonts and gametocytes from bone marrow smears (13)
Kenya	Trophozoites compact frequent multiple infection and great enlargement of host cells stippling often absent parasites sometimes extra corpuscular—the so-called *P wilsoni* Roberts 1940 (14)
Tanganyika	Multiple infection and distortion of host cells compact trophozoites and schizonts (15)
W Africa	Ovale like forms (16)
United States	Occasional segmenters with few merozoites in St Elizabeth strain (10) band forms in *P vivax* presumably indigenous (17)
Various	Ovale like morphology related to repeated artificial passage by blood inoculation (18) (19) small schizonts with fewer merozoites in chronic infection (19) (20) strain variations in number of merozoites (21)

Morphological changes in *P vivax* may also have an artificial cause Slow drying in thin blood films favours a clean cut signet ring formation in the young trophozoites quick drying tends to perpetuate the amoeboid form of the living parasite Schuffner s dots are sensitive to changes in staining particularly to pH (plate XXXVIII) while parasites damaged by drugs may show all degrees of cytoplasmic fragmentation and failure to stain

DOUBTFUL SPECIES OR VARIETIES

Though abnormal forms of *P vivax* are fairly common only three vivax like organisms seem to have been regarded as distinct species or sub-species—*P vivax* var *minuta* Emin 1914 *P wilsoni* (Roberts 1940) and *P ovale* Stephens 19-- *Plasmodium ovale* is now generally regarded as a valid species the other two organisms have a doubtful taxonomic status

Plasmodium vivax var minuta Emin 1914—A malaria parasite of uncertain identity has been described by Emin (1914) Found in the blood of six pilgrims from the island of

Young M D and Eyles D E (1949) *J nat Malar Soc* 8 9
Chalmers, A J and Archibald, R G (19) *J trop Med (Hyg)* 23 33
12 Emin A (1914) *Bull Soc Path exot* 7 385
13 Yofe M (1948) *Trans R Soc trop Med Hyg* 4 99
14 Roberts, J I (1940) *E Afr med J* 17 212
15 Wilson Margaret (1949) *Trans R Soc trop Med Hyg* 43 97
16 Macfie, J W S and Ingram A (1917) *Ann trop Med Parasit* 11
17 Lawson Mary R (1916) *J exp Med* 24 29
18 Missiroli A and Sinscalchi R (1949 *Riv Malariol* 8 231
19 Ciovannola A (1935) *Amer J trop Med* 15 73
20 James, S P *Malaria at Home and Abroad* John Bale Sons Danielsson London 1920
21 de Buck A (1935) *Arch Schiffs- u Tropenhyg* 39 342

Camaran in the Red Sea the parasite seemed to Emin to be a small form of *P vivax*, and he gave it the name *P vivax* var *minuta* Some of Emin s films were examined by Ziemann (1915) who thought that the parasite more closely resembled *P falciparum*, and proposed the name *P camaranense* The original drawings of Emin are reproduced in plate XX From the evidence of the drawings and descriptions we are disposed to think that neither name is justified and that the parasite is in fact *P falciparum*

Plasmodium wilsoni (Roberts, 1940)—This parasite found in blood films from Abyssinian refugees and among various East African races during an epidemic is a vivax like organism with peculiar features which seemed to Roberts (1940) to justify the creation of a new species He gave it the name *P wilsoni*, a similar parasite having been seen by Margaret Wilson in 1937 in three cases infected in the Moshi district of Tanganyika (Wilson 1949) The main features of the parasite and of the infections to which it gives rise, are summarised below

(*a*) The attacks are severe with a repeated relapses in spite of intensive quinin and atebrin treatment and the infections are very heavy

(*b*) The young trophozoites are compact and non amoeboid, resembling *P ovale* multiple infections are common often three or four to a cell, band forms were not observed

(*c*) The older trophozoites are compact, with a well defined vacuole, the pigment granules are not aggregated the parasites are often extra corpuscular

(*d*) The schizonts contain from 12 to 19 merozoites

(*e*) The gametocytes are pale, with well distributed pigment the pale blue cytoplasm of the female is said to be markedly different from that of *P vivax*

(*f*) The host cells are always enlarged, sometimes to a much greater extent than the vivax infected cell, stippling is often absent but there may be a faint quartan like stippling in some infected cells and a trabecular appearance in others the cells are usually decolourised

A somewhat similar parasite has been described in Malaya by Field (1942) who believed the Malayan organism to be a random variant of *P vivax*

The validity of *P wilsoni* as a new species has not been generally accepted There is no evidence that the peculiar characteristics described are constant after mosquito passage to new hosts and until this proof of specific identity has been established the parasite should perhaps be regarded as an aberrant form of *P vivax*

ZIEMANN H 19 5 (cf p 52)
ROBERTS, J I (1940) A n w malaria paras te of man n East Africa *E Afr med J* 17 212
WILSON MARGARET (949) Abnormal f rms f *Plasmod um vi ax* *T R Soc t p M d Hyg* 43 97
FIELD J W (1942) Morphological variati n in *Plasmod um a G* and F letti 1890 *Parasi l gy* 34 8

CHAPTER V

PLASMODIUM MALARIAE (GRASSI AND FELETTI 1890)

The parasite *Plasmodium malariae* is the cause of quartan malaria The organism is distinguished from other species infecting man by its morphological characters by its slow development in both human and insect hosts and by its capacity for prolonged survival in the human host The infections are usually mild with paroxysms which tend to recur every third day in step with the 72 hour cycle of the organism *

HOST AND HABITAT

Parasites closely resembling *P malariae* are found in monkeys The quartan like parasite *P inui* is common in Indian and Malayan macaques and natural infections with a similar organism occur in African chimpanzees The quartan like parasites of chimpanzees can be transmitted to man while chimpanzees can be infected with *P malariae* of human origin, on these grounds Rodhain (1948) believes that the parasites are the same and that the chimpanzee of Central Africa constitutes a natural reservoir of quartan infection

Under experimental conditions *P malariae* can be passed through mosquitoes to man only with difficulty and mainly on this account its life cycle in the human host has not been fully defined There is good reason to believe that the organism develops in the parenchymal cells of the liver before invading the blood Garnham having demonstrated a pre-erythrocytic liver phase in *P inui* a quartan like parasite of monkeys and it is possible that persisting exo-erythrocytic parasites in the liver or other fixed tissues form a latent reservoir during chronic infection Raffaele (1946) has seen parasites which he believes to be exo-erythrocytic forms of *P malariae* in the bone marrow a few days after the inoculation of sporozoites and as long as one year later

BEHAVIOUR

The distinctive features of *P malariae* are the low infectivity for mosquitoes the prolonged sporogonic and schizogonic cycles the low density in the blood and the occasional life-long persistence in the human host Quartan infections, for these reasons are the least common of the malaria infections in most countries they have a long incubation period and they tend to have a protracted course They seldom endanger the life of the host but for reasons unknown they are liable to cause damage to the kidneys

GEOGRAPHICAL RANGE

The development of *P malariae* in mosquitoes is possible when the average temperature does not fall below 15 C (59 F) The organism has been found between the latitudes 53 North in the Netherlands and 30 South in Argentina with a distribution which tends to be patchy often bearing little obvious relationship to the prevalence of mosquitoes which readily transmit other species In the Nile Delta for example *P malariae* is absent though it is the most common species in some of the oases in the Egyptian Sahara and there are scattered quartan foci in the Near East India Ceylon Malaya and elsewhere In Malaya quartan infections are not commonly found in hospital patients—less than five per cent of the admissions to government hospitals in recent years—but there are localised rural areas where *P malariae* is the dominant species

* Malarial infections are usually designated by the specific name of the parasite Infections due to *P falciparum* or *P ovale* are thus known as falciparum malaria or ovale malaria. The logical name for quartan infection is malariae malaria but this name is repetitious and it may and the old and more euphonious name quartan malaria has resisted the pressure towards consistency

Rodhain J (1948) Susceptibility of the chimpanzee to *P malariae* of human origin. *Amer J trop Med* 28 639.
Raffaele G (1946) Modern views on the biology of the malaria parasites *Acta Tropica*, Basle 3 29 (Abstract *Trop Dis Bull* 1946, 43 6)

For this peculiar distribution we have no complete explanation Gametocyte production in quartan infections is low and sporadic, and the sluggish development in mosquitoes implies a considerable hazard to the organism We may infer that the sporogonic cycle is highly vulnerable



Fig 22—PROPORTION OF QUARTAN INFECTIONS IN PATIENTS ADMITTED FOR MALARIA TO THE GOVERNMENT HOSPITALS OF SELANGOR PERAK, NEGRI SEMBILAN AND PAHANG, 1930-1954

to unfavourable circumstances, and that the organism survives in nature only when the vector mosquitoes are long lived Discounting residual infections in the human host, we may also infer that the organism would probably be the first to disappear as a result of mosquito control The apparent recession of quartan malaria in Malaya over the past few years lends support to this inference Residual insecticides introduced in a small way for malaria control soon after the second world war have since been used on an increasing scale Meanwhile the number of patients admitted to government hospitals with quartan malaria has shown a marked and apparently selective reduction, so much so that there is at least a hope that *P malariae* may be a vanishing species Twenty five years ago, in 1930 838 patients were treated in the government hospitals of the Federated Malay States for quartan malaria, in the same hospitals only 10 quartan infections were treated in 1954

Table 20—MALARIA ADMISSIONS TO THE GOVERNMENT HOSPITALS OF SELANGOR, PERAK PAHANG AND NEGRI SEMBILAN, 1930-1954

Year	Malariae	Falciparum	Vivax	Year	Malariae	Falciparum	Vivax
1930	838	16,080	7 570	1947	138	4 134	[illegible]203
1931	745	9,529	5 010	1948	85	2 970	1,[illegible]00
1932	371	7,7[illegible]1	3 192	1949	56	2 606	981
1933	4[illegible]9	8,831	3 261	1950	31	2 5[illegible]9	672
1934	364	7 599	3 488	1951	23	3 152	855
1935	441	11,333	4 497	1952	16	2 837	830
1936	344	9 895	4 403	1953	15	2 754	905
1937	381	12 715	6 040	1954	10	2 180	711
1938	500	17,747	8 549				

The marked change in the apparent incidence and relative prevalence of quartan malaria in Malaya is well shown in the figures of admissions for malaria to the government hospitals of Selangor Perak, Negri Sembilan and Pahang the former Federated Malay States The figures are drawn from the Annual Reports of the Medical Department and from returns submitted to the Malaria Advisory Board of the Federation

Why *P malariae* should sometimes and in some ar as be the dominant species is less readily explained Quartan infections tend to be long lasting in the human host and a high quartan incidence may be a legacy of past events During periods of brisk transmission involving all species *P falciparum* takes the centre of the stage succeeded after a few months by *P vivax* Thereafter *P malariae* masked at first by mixed infections and a low density in the human host comes to the fore as other species with their shorter life are on the wane We might thus explain a high quartan incidence as a residual infestation depending on a spell of active transmission in the past and a run of years with transmission in abeyance or at low ebb But there are other possibilities and the epidemiology of quartan malaria seems still to hold an element of mystery

Knowles and Senior White (1930) believe that *P malariae* is an old and senescent species the oldest of the malaria parasites of man The grounds for this belief are

(i) the low reproductive capacity in nature suggests a disappearing species

(ii) the spotty distribution of *P malariae* all over the world and the apparent special association of the organism with primitive peoples suggest that the present quartan foci represent a residual infestation by a once widespread species

(iii) the wide distribution in small mammals of parasites resembling *P malariae* suggests that the species is much older than man in the evolutionary scale by contrast mammalian species resembling *P falciparum* have a restricted zoological range

(iv) the low grade infections in man suggest an ancient symbiosis

Speculating on the evolutionary origin of the malaria parasites of man they suggest that the first of the malaria parasites adapted to man arising in the ancient cradles of the human race was *P malariae* the second was probably *P vivax* a species which has followed man into higher latitudes and altitudes than other species the third and most recent was *P falciparum* which from the severity of the infections in man is still in the course of evolutionary adaptation

INCUBATION PERIOD

Under experimental conditions quartan infections are transmitted through mosquitoes to man only with great difficulty Comparatively few mosquito-induced infections from bites on known dates have been recorded and our knowledge of the duration of incubation is hence based on meagre evidence Data from various sources on the intrinsic incubation period in twenty four mosquito-induced infections have been summarised by Shute and Maryon (1951) in two infections only was the incubation period less than 20 days in all others the period of incubation varied between 21 and 59 days

SCHIZOGONY IN THE RED BLOOD CORPUSCLES

The periodic clinical phenomenon of quartan malaria are related as in all forms of human malaria to the cyclic development of the parasites in the red blood corpuscles Golgi working in Padua where the quartan fevers were common was the first to recognise this relationship The name Golgi cycle sometimes used for the schizogonic cycle of the malaria parasites in the blood is a fitting reminder of this great discovery

The first invasion of the blood cells by *P malariae* initiating the cycle is presumed to come from parasites which have developed in the fixed tissues By analogy with *P falciparum*

KNOWLES, R. AND SENIOR WHITE R (1930) Studies in the Parasitology of Malaria. *Memoir No 18 Indian J med Res*

SHUTE P C AND MARYON M (1951) Studies in the transmission of *Plasmodium malariae* by *Anopheles* mosquitoes *Parasitology 41* 9

and *P vivax* we may assume that there is a pre erythrocytic phase in the liver parenchyma—and possibly in other fixed tissues—but proof of this assumption has still to come

The erythrocytic cycle lasts about 72 hours and the paroxysms of fever associated with the liberation into the blood stream of the merozoites of a single brood occur at intervals of three days Sometimes the cycle takes a little less than 72 hours sometimes a little more the paroxysms then occur earlier or later the clinical phenomenon known as *anticipation* or *retardation* Young *et al* (1940) have drawn attention to the curious relationship between the time of the paroxysms and the activity of the subject Most paroxysms occur about noon When however, the hours of activity and sleep in the subject are reversed the paroxysms gradually become nocturnal

The pattern of the temperature is a clinical expression of the number of broods and the timing of their segmentation A single brood gives rise to a simple quartan fever, two broods to a double quartan with paroxysms on two successive days with one fever free day and three broods to a triple tertian (quotidian) fever with a paroxysm every day Boyd (1940) has studied the fever pattern in a series of 40 infections attacks usually began and ended with a simple quartan fever from a single recurring cycle, all three patterns might be found in a single attack though occasionally the fever was quartan throughout In a series of 100 quartan infections observed for brief periods in Kuala Lumpur the fever was quartan in 27 double quartan in 5 and quotidian irregular or absent in the remainder

Fever threshold—Parasites in quartan infections are usually present in the peripheral blood in detectable numbers on the first day of fever Kitchen (1949) in a series of 90 infections representing four strains of *P malariae* failed to find parasites at the onset of symptoms only on one occasion the interval between the first appearance of parasites and the first day of fever averaged eight days, with a range of one to forty seven days This relative tolerance for *P malariae* on the part of the host is also evident from the number of parasites in the blood at the onset of symptoms Data from 26 quartan infections recorded by Boyd (1940) show that the fever threshold ranged from 10 to 3 000 parasites per c mm of blood The higher threshold densities were associated with a prolonged incubation period and with a longer time lag between the recognition of parasites and the onset of fever

Parasite density—Quartan infections tend to be the mildest of the malaria infections of man Their benign course is probably related to the low rate of increase to the low peak of parasitaemia even in untreated infections and to the rarity of dangerous accumulations of parasites in vital organs The low parasite density prevailing in most quartan infections has been attributed to the prolonged schizogonic cycle and to a possible selection of the older erythrocytes by the merozoites

The parasite densities recorded in quartan infections have usually been less than 5 000 sometimes more than 25 000 and very rarely over 50 000 per c mm of blood Ciuca and his colleagues (1934) in a study of 294 infections artificially induced by two strains of *P malariae*

YOUNG M D STUBBS T H AND COATNEY G R (1940) Studies on induced quartan malaria in negro paretics II The effect of modifying the ... conditions *Amer J Hyg* 31 Sect C, 63

BOYD M F (1940) Observations on naturally and artificially induced quartan malaria *Amer J trop Med* 20 749

KITCHEN S F Quartan malaria Chapt 42 in *Malariology* Ed M F Boyd W B Saunders Co London and Philadelphia, 1949

CIUCA M BALLIF L AND CHELARESCU VIERU M (1934) Cited by Brumpt E Chap 4 in *Malariology* Ed. M. F Boyd, W B Saunders Co London and Philadelphia 1949

of West African and Italian origin found that in only two per cent of their cases was there more than one parasite in every two fields Kitchen (1949) states that his patients seldom attained a parasite density of ˌ0 000 per c mm of blood he records however an exceptionally intense infection with a parasite count of 114 000 per c mm two days before death

Table 21—PARASITE DENSITY ON THE FIRST DAY OF TREATMENT IN 171 QUARTAN INFECTIONS NATURALLY ACQUIRED IN MALAYA

	Parasite counts per c mm blood				
	Less than 5 000	5 000-25 000	25 000-100 000	Over 100 000	Total
Number	127	41	3	0	171
Percentage	74	-4	-	—	—

Counts made on the first day of treatment in 171 quartan infections observed by the Malaria Research Division of this Institute are summarised in table 21 The parasite density in

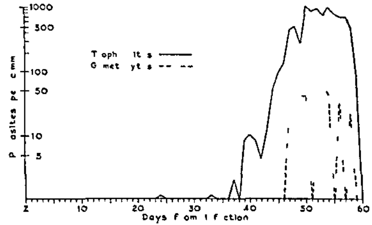


Fig 23—TROPHOZOITE AND GAMETOCYTE WAVES IN A SPOROZOITE INDUCED QUARTAN INFECTION OF SOUTH WEST PACIFIC ORIGIN ILLUSTRATING THE LONG INCUBATION PERIOD AND THE LOW PARASITAEMIA THE INFECTION WAS TREATED ON THE FIFTY SIXTH DAY (after Mackerras and Ercole 1948)

three-quarters of these infections was less than 5,000 per c mm of blood and in three infections only did the count exceed 25 000 per c.mm the highest count recorded was 30 000 per c.mm There were no deaths in the series but 11 patients had a severe albuminuria

MACK RRAS, M. J AND ERCOLE Q N (948) Observations on the lif cycle of *Plasmodium malariae* *Aus J* *Biol. med* 5 76 5 5

Distribution in the host—The complete schizogonic cycle of *P malariae* occurs in th peripheral blood and an even distribution of the organisms throughout the circulatory system at all stages of the cycle seems to be usual Marchiafava and Bignami state that the forms found

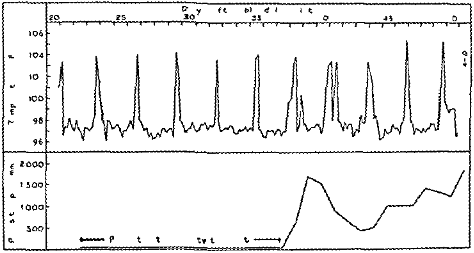


Fig 24—BLOOD INDUCED INFECTION WITH *Plasmodium malariae* OF MALAYAN ORIGIN WITH PROLONGED QUARTAN FEVER (Horton Case M P March 1955)

in spleen smears are not notably different from those in capillary blood from the finger Even so the possibility of a dangerous localisation in the inner organs cannot altogether be dismissed Leger and Ryckewaert (1917) record a fatal infection in which the parasites were numerous in the bone marrow heart muscle, and brain though comparatively few in the blood

Duration of infection—Clinical activity in untreated or under treated quartan infections typically lasts for several months, usually with several remissions Boyd (1940) found the mean duration of naturally induced attacks to be a little over four months but the organism may persist in the body for many years even in rare cases for the life time of the host Evidence on this point comes mainly from the quartan relapses years after the first attack which have been reported and from the infections transmitted during blood transfusions from donors who have had quartan malaria in the past Shute (1944) found quartan parasites in the blood of a woman who had lived in England for 21 years, the infection having apparently been acquired in Calcutta where she had lived as a child eleven years later in 1954 thirty two years after the original infection there was a further recurrence of fever with quartan parasites in the blood Where the parasite lies quiescent in these cases whether in the fixed tissues or in the blood is not known but the transference of quartan infection by blood transfusion from donors infected years before suggests that infection may persist as a low grade symptomless parasitaemia An interesting case is recorded by Kitchen (1949) a patient was infected experimentally with *P malariae* in 1933 twelve years later blood from this patient was transfused to another patient twenty one days after the transfusion quartan parasites were found in blood films from the recipient

LEGER M AND RYCKEWAERT F (1917) Hémat z air d l fièvr quarte et acc pernicieux mo l *Bull Soc P* x i 10 879
BOYD 1940 (f p 70)
SHUTE P G (1944) R lap f quartan f aft 12 and 21 y ars *Lancet* 145
KITCHEN 1949 (f p 70)

The risks remote though sometimes tragic, of blood transfusion from donors who have lived in quartan endemic areas is emphasised by the case recorded by Nabarro and Edward (1930) A child in England received an intramuscular injection of blood from her father who had lived in Ceylon The child died from quartan malaria ten weeks later The father had left Ceylon twelve years before and had never to his knowledge had malaria but an injection of his blood produced a quartan infection in a paretic undergoing malario-therapy at the Horton Centre

GAMETOGONY

Gametocyte production in quartan malaria tends to be low and sporadic The young forms like those of *P falciparum* and *P vivax* develop in the deep organs and do not normally enter the peripheral blood They closely resemble young trophozoites and in blood films they may easily pass unrecognised as gametocytes In primary attacks the adult gametocytes enter the circulation at any time from a few days to a few weeks after the first detection of schizogonic forms in relapses they tend to appear somewhat earlier Thereafter in untreated or under treated infections they may persist for many weeks usually at a low density Ciuca *et al* (1934) report that only in one third of their quartan patients were male and female gametocytes found in the same blood films a further weakness in gametogony to which the difficulties in the natural transmission of the organism are doubtless related

VARIATION

The occurrence of races or varieties of *P malariae* differing in form or behaviour has been suggested but the evidence for this view is somewhat meagre Brumpt (1949) states that there are biological differences as some strains fail to immunise against others while Cruz (1915) believed that a special kind of *P malariae* was responsible for the quartan oedema of the Amazon Valley The only reports of morphological variations known to us are those of Garnham (1936) Guimaraes and Cortez (1919) and Stephens (1915)

The quartan parasites prevalent in a native reserve in Kenya differed according to Garnham in their morphology from the usual forms A tenue appearance was common in the rings trophozoites which were two-thirds grown had a large vacuole and a well-defined peripheral thickening, the division of the chromatin in the schizonts might be delayed until a few hours before they reached complete maturity and the pigment was golden yellow rather than black Some of these features an occasional tenue form in the young trophozoites a persisting vacuole in the large trophozoites and yellowish pigment are common in the Malayan organism (plate XXVIII)

The abnormal features reported by Guimaraes and Cortez were found in a single case The parasites were said to differ from typical *P malaria* in their great numbers in the rarity of gregariniform schizonts (band forms) and in the abundance of adult schizonts of rounded form

Stephens (1915) described and illustrated the unusual parasites found in a single film from an African child on the Gold Coast Many of the young trophozoites showed a marked variation in the form of the chromatin and cytoplasm rather like the tenue forms of

N D ND EDW D D G H (939 A al al transmi sion of malaria child by the i jection of blood *Lan* 556

Ciuca *I* 934 (f p 70)

Br mp E 1949 (ef p 8)

G NHAM P C C (936) Hyperendemic malaria in na ve reserve of Ke y and the influence pon i course f at brin and plasmoq ine *T R Soc trop M d Hyg* 29 6

Gu MAR S, A G ND CORTEZ M (9 9 U caso d Quartan no E tado S Paulo (Abstract *Trop D B ll* 9 15 26

Cr z O (9 5 *Brazil Med* 29 345 (Abst act *T op Dis Bull* 9 6, 7 9)

STEPHENS J W W (9 5 On he peculiar morphological ppearances of malaria paras *Ann t p Med Parasit* 9 169

P falciparum There were normal quartan parasites in the film The author, suggesting (*a*) that the bizarre appearances may have been produced artificially during the preparation of th film (*b*) that the parasites may have been degenerate or (*c*) that they may have represented a new species appears to favour the last view To-day, the creation of a new species on evidence of this kind would scarcely be thought justified

Whether the apparent rarity of morphological variation in *P malariae* reflects the true position or whether variations occur which have not been recognised is not known. Quartan infections are far less common in most countries than falciparum and vivax infections they have been less studied and abnormal features might easily be missed in the low grade infections which are usual *We are disposed to think, however, that the morphology of P malariae is* relatively constant, that its low reproductive capacity is unfavourable for the evolution of distinctive varieties, and that the sluggish activity of the trophozoites does not lend itself to the whimsical variations in form sometimes found in *P falciparum* and *P vivax*

CHAPTER 11

PLASMODIUM OVALE STEPHENS 1922

The parasite *Plasmodium ovale* is the cause of ovale malaria The infections are usually mild and have a tendency to spontaneous recovery They are characterised clinically by regular tertian or quotidian paroxysms which are usually less distressing than those of vivax malaria and seldom exceed ten in number even in the absence of treatment

The parasite was discovered by Stephens (1922) in the blood of a British soldier who had returned to England from East Africa in January 1918 The soldier had had several attacks of malaria while abroad four months after his return to England he had another attack and parasites thought to be *P vivax* were found in blood films He had a further attack in July 1918 when the unusual form of some of the parasites led Stephens to believe that they represented a new species to which he gave the name *Plasmodium ovale* from the oval shape of many of the infected cells * Stephens and Owen (1927) found the same parasite in a patient from Nigeria and Yorke and Owen (1930) using the parasite in the practice of malario-therapy proved that the morphological characters were constant after direct passage by blood inoculation to fresh hosts In 1931 Yorke sent blood from a patient infected in the Belgian Congo to the Horton Malaria Laboratory Epsom a patient was infected by blood inoculation and from this source a batch of English *Anopheles maculipennis* (*atroparvus*) was infected (James Nicol and Shute 1932) These mosquitoes transmitted the disease when fed on non immune subjects with no change in the morphological characters of the organism

HOST AND HABITAT

Parasites with a close resemblance to *P ovale* have been found only in man Attempts to transmit the organism to monkeys both by blood inoculation and by mosquito bites have failed (Shute 1936) Recent work by Garnham *et al* (1954) has shown that the sporozoites of *P ovale* like those of *P falciparum* and *P vivax* enter the parenchymal cells of the liver the organisms complete their development in the liver in about nine days and a day later they appear in the blood Relapses in ovale malaria are unusual and for this reason the occurrence of a secondary cycle in the liver is thought to be unlikely though the possibility cannot be excluded

GEOGRAPHICAL RANGE

Since Stephens described and named *P ovale* many ovale infections have been reported mostly among Europeans returning from Africa and it seems that tropical Africa is the main region of natural distribution The parasite has also been reported from South America

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* Parasites resembling *P ovale* had been found [illegible] by Craig (1900) in the Philippines and by Macfie and Ingram (1917) West Af[rica]

STEPHENS, J W W (1922) A new malaria parasite of man *Ann trop Med Parasit* 16 383

CRAIG C F (1900) Cited by Craig (1914)

CRAIG C F (1914) New varieties and species of malaria plasmodia. *J Parasit* 1 85 (Abstract *Trop Dis Bull* 1915 5 249)

MACFIE J W S AND INGRAM A (1917) Observations on malaria in the Gold Coast Colony West Africa *Ann trop Med* 11

STEPHENS, J W W AND OWEN D U (1927) *Plasmodium ovale* *Ann trop Med Parasit* 21 293

YORKE W AND OWEN D U (1930) *Plasmodium ovale* *Ann trop Med Parasit* 24 593

JAMES, S P NICOL W D AND SHUTE P G (1932) *Plasmodium ovale* Stephens passage of the parasite through mosquitoes and successful transmission by their bites *Ann trop Med Parasit* 26 139

SHUTE P G (1936) Unpublished data

GARNHAM P C C, BRAY R S, COOPER W, LAINSON R, AWAD F I AND WILLIAMSON J (1954) Pre-erythrocytic stages of human malaria *Plasmodium ovale* A preliminary note *Brit med J* 1 257

(Muhlens 1934) New Guinea (Jackson 1944) the Middle East (Adler and Zuckerman 1952), Asia (Muhlens 1938) and Eastern Europe (Eskin, 1938) More recently Jeffery and his colleagues (1954) have found a similar organism in an American serviceman infected in the Pacific, either in the Philippines or in the Admiralty Islands They have called it the Donaldson strain Their detailed morphological study of the organism in man and mosquito leaves little doubt that in all essential respects the Donaldson strain resembles *P ovale* of African origin

Though *P ovale* undoubtedly occurs in regions outside its main endemic home in tropical Africa the identity of some of the parasites described as *P ovale* from other parts of the world has not been established Variant forms of *P vivax* may closely resemble *P ovale* and the proof of identity will usually depend on the transmission of suspected *ovale* through mosquitoes to fresh hosts

INCUBATION PERIOD

Little is known of the incubation period of naturally acquired ovale malaria, but there is reliable information from the infections which have been induced by mosquito bites under controlled laboratory conditions At Horton the mean incubation period in 36 primary infections was 13 6 days with a variation between 11 and 16 days Infections with the Donaldson strain seem to have a slower onset, Jeffery and his colleagues (1954) having found the mean incubation period in 37 mosquito-induced infections to be 17 7 days with a range of 14 to 20 days As in vivax infections the parasites appear in blood films at about the time when the host reacts with fever, sometimes a day or two earlier or later

LATENCY

There is evidence that ovale infections sometimes remain latent for months Among the 36 infections observed at Horton by James *et al* (1949) was a single infection with an incubation period of 85 days, a prolonged incubation of 19 months has been reported by Manson Bahr and Muggleton (1945), and it is interesting to note that the Donaldson strain was originally recovered several years after the carrier, Donaldson had left the region where he had presumably acquired the infection In this respect, as indeed in certain other respects, the parasite resembles *P vivax* Where the organism survives in the host during periods of latency whether in the blood or in the fixed tissues is not known

CLINICAL COURSE OF INFECTION

Infections with *P ovale* resemble vivax infections but they are milder, with a shorter clinical course and a tendency to an earlier spontaneous recovery The fever may be irregularly remittent quotidian, or tertian A remitting fever is common at the onset but in the developed disease the fever is usually quotidian or tertian in type Sometimes the fever may be quotidian throughout A peculiarity of the paroxysms is that they often begin late in the evening rather than at midday as is usual in vivax malaria Typical rigors in which the teeth chatter and the

MUHLENS, P (1934) Ueber Plasmodium ovale (Stephens) *Arch f Schiffs u Trop-Hyg* 38 367

JACKSON A V (1944) *Plasmodium ovale* malaria a report of two cases contracted in New Guinea *Med J Austral* 2 8

ADLER S AND ZUCKERMAN A (1952) Observations on the morphology of a strain of *Plasmodium ovale* *Ann trop Med Parasit* 46 89

MUHLENS P *Die Krankheiten und Hygiene der Warmen Lander* Georg Thieme Leipzig 1938

ESKIN V A (1938) A case of natural infection with *P ovale* man from U S S R *Med Parasit Moscow* 7 40

JEFFERY G M YOUNG M D AND WILCOX A (1954) The Donaldson strain of malaria History and characteristics of the infection in man *Amer J trop Med Hyg* 3 628

WILCOX A JEFFERY G M AND YOUNG M D (1954) The Donaldson strain of malaria 2 Morphology of the erythrocytic parasites *Ibid* 3 638

JEFFERY G M AND YOUNG M D (1954) The Donaldson strain of malaria 4 An evaluation and status *Ibid* 3 660

JAMES S P NICOL W D AND SHUTE P G Ovale malaria Chapter 44 in *Malariology* Ed M F Boyd W B Saunders Company London and Philadelphia 1949

MANSON BAHR P AND MUGGLETON W J (1945) The persistence of *Plasmodium ovale* *Brit med J* 369

patient shivers from head to foot are less frequent than in vivax infections, and they are of shorter duration lasting only for about half an hour compared with an hour or more in vivax attacks Whether the rigors are severe mild or absent seems to depend as in vivax malaria

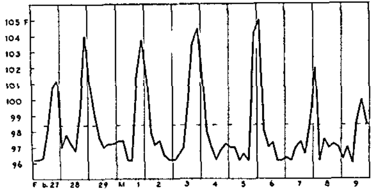


Fig 25—PRIMARY SPOROZOITE INDUCED OVALE INFECTION WITH TERTIAN FEVER THIS WAS THE FIRST PATIENT OF THE HORTON OVALE SERIES INFECTED BY MOSQUITO BITES A SECOND PATIENT INFECTED BY THE SAME MOSQUITOES RAN A QUOTIDIAN FEVER THROUGHOUT (after James *et al* 1932)

on the numerical prevalence of segmenting schizonts in the peripheral circulation at a given time (We may perhaps mention in parenthesis that rigors are unusual in falciparum and quartan malaria a point of some importance as th re is a tendency even among medical men to rule out malaria in fever patients who have recently returned to Europe from the tropics if the fever is unassociated with rigors)

RELAPSES

Infections due to *P ovale* unlike vivax infections seldom relapse No relapses were observed among the 36 patients infected by mosquito bites at Horton though in 14 patients the primary attack was untreated and in no case was any antimalaria drug given after the primary attack Jeffery Young and Wilcox (1954) record only two relapses in 38 infections sporozoite induced with the Donaldson strain though Garnham *et al* (1955) report that two of the three subjects infected by mosquito bites in the course of their investigations on the pre-erythrocytic development of *P ovale* each had relapses of infection on two occasions In this direction *P ovale* contrasts sharply with *P vivax* relapse rates up to 80 per cent or more having been reported in vivax infections One possible explanation of this difference is that primary ovale infections tend towards a spontaneous recovery after six to eight peaks of fever, suggesting that immunity to *P ovale* is quickly and permanently acquired and there is of course the further possibility that *P ovale* is less prone than *P vivax* to persist in the fixed tissues

Infections with *P ovale* have sometimes declared themselves in England apparently for the first time some months after the subjects have returned from Africa We are inclined to regard these clinical manifestations as delayed primary attacks though the chance that they are relapses can seldom be excluded

GARNHAM P C C, BRAY R S, COOPER W, LAINSON, R, AWAD F I AND WILLIAMSON J (1955) The pre-erythrocytic stage of *Plasmodium ovale* *Trans R Soc trop Med Hyg* 49 158

MALAYA No 34 1955

GAMETOGONY

The gametocytes of *P ovale* may first appear in the peripheral blood as early as the fourth day or as late as the eighteenth day, after the first confirmation of parasitaemia (James *et al* 1949) Often they disappear after a day or two though they may persist for a week or more Seldom are they numerous and only on rare occasions in the Horton series did the mosquitoes fed on gametocyte carriers show more than ten to twenty oocysts There is evidence however that gametocytes may be less elusive in countries where ovale infection is endemic, Wilson *et al* (1935 1936) have found that in Tanganyika ovale gametocytes may be more numerous in children than the gametocytes of other species As with *P vivax* the female gametocytes persist in the blood for a longer period than do the males

VARIATION

Differences of behaviour in strains of *P falciparum* and *P vivax* of differing geographical origin are well known The English mosquito *A maculipennis atroparvus* is susceptible to infection with *P falciparum* of Roumanian origin but refractory to certain tropical strains patients at Horton who were immune to a Madagascan strain of *P vivax* have been found susceptible to a strain from the French Cameroons These are but two of many examples of biological variation in *P falciparum* and *P vivax* Few such variations have been reported in *P ovale* No immunological or other biological differences have been recognised at the Horton centre in *P ovale* from the Belgian Congo, Nigeria Sierra Leone, the Gold Coast and elsewhere in West Africa The morphology of *P ovale* seems to be similarly free from marked variation No differences of any note have been seen in the twelve strains* used at the Horton Centre and most accounts of the organism correspond broadly with the original description of Stephens An exception is the report by Hauer (1937) of morphological changes of an ovale strain which had been maintained by blood passage at the Hamburg Institute of Tropical Diseases Abnormal forms with a greater enlargement of the host cell and up to 16 merozoites in the mature schizonts began to appear at the 22nd passage at the 38th passage there was a tendency to revert to the original form A similar tendency to produce up to 16 merozoites has been reported by Garnham *et al*, 1955

The comparative harmlessness of *P ovale* its fleeting appearance in the blood stream and its rarity suggest that *P ovale* may be an ancient species approaching extinction (Wilson and Wilson 1935) By this assumption too we may perhaps explain the morphological stability of the organism

THE SPECIFIC STATUS OF PLASMODIUM OVALE

Most workers in malaria are agreed that *P ovale* despite its resemblances to *P vivax* has inheritable differences in morphology and behaviour and that these differences justify an independent specific status The evidence in support of this view comes mainly from the work of James and his colleagues at the Horton Centre where *P ovale* has been used for malariotherapy since 1931 and some 366 patients have been infected with parasites derived from

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* Th[illegible] used h[illegible] to imply [illegible] logically homogeneous

WILSON D B AND WILSON MARGARET (1935) Infection with *Plasmodium ovale* Stephens in Tanganyika Territory *Trans R Soc trop Med Hyg* 28 469

— — (1936) Rural hyperendemic malaria in Tanganyika Territory *Ibid* 29 583

HAUER A (1937) [illegible] Beobachtungen an einem *Pl ovale*-Stamm *Arch Schiffs u Tropenhyg* 41 153

JAMES *et al* 1949 (cf p 76)

GARNHAM *et al* 1955 (cf p 77)

varied sources James *et al* (1935) have summarised the reasons for regarding *P ovale* as a valid species

1 the distinctive morphology is not changed on passage from person to person
2 the form of the parasite is maintained through mosquito passage
3 the pigment arrangement in the oocyst is distinctive
4 the parasite is immunologically distinct, persons who are immune to *P vivax* have been infected with *P ovale*
5 the clinical course of primary ovale infection is different from that of primary vivax infection

We may add the further observation of Garnham *et al* (1955) that the pre-erythrocytic schizonts of *P ovale* are different in size and shape from those of *P vivax*

The specific status of the parasite has been contested on grounds which are reviewed by Giovannola (1935) Under particular conditions *P vivax* may closely resemble *P ovale* so much so that in blood films the one can scarcely be distinguished from the other One example of this ovale like morphology in *P vivax* is the Wagner Jauregg strain which was isolated in 1919 in the Balkans and maintained by interhuman transfer after 14 years of passage from man to man the strain had acquired the main morphological characters of *P ovale*

1 The young trophozoites assumed a compact form at an early stage
2 Enlargement of the host cells was slight
3 The nuclei divided prematurely and the schizonts averaged 13 merozoites against 19 in a strain received from James
4 The gametocytes were small and scarce

On these grounds and on similar evidence from others who had used *P vivax* for malariotherapy, Giovannola regarded *P ovale* as a modification of *P vivax* produced by long continued asexual propagation To most malariologists however the constant differences between *P vivax* and *P ovale* not only in form but also in the patterns of human infection have seemed to be good reasons for regarding *P ovale* as a separate species and there is no doubt on the convenience of the separation

Shute and Maryon (1954) recalling a suggestion of the late Dr Wenyon that *P ovale* may be a hybrid draw attention to the possibility that *P ovale* may represent a cross between *P vivax* and *P malariae* For the questions raised by this interesting view there is no present answer

JAMES, S P, NICOL, W D AND SHUTE, P G (1935) The specific status of *Plasmodium ovale* Stephens *Amer J trop Med* 15 187

GIOVANNOLA, A (1935) *Plasmodium ovale* considered as modification of *Plasmodium vivax* after long residence in the human host *Amer J trop Med* 15 175

GARNHAM *et al* 1955 (ref p 7

SHUTE, P G AND MARYON, M (1954) Observations on *P ovale* *Proc 5th Congr Trop Med & Malar* I bul 1953 2 44

CHAPTER VII

METHODS OF EXAMINATION

The examination of the blood is the one certain method of diagnosis—the only manner in which a positive diagnosis of malaria *is to be made* (Thayer, 1900) The newer knowledge of the life cycles of the malaria parasite in the human host has done little to weaken this positive statement From the examination of the blood and from this examination alone can we normally make a positive diagnosis in the patient sick with malaria The blood may be got from varied sources, it may come from the superficial capillaries, the arteries or the veins from the clot in a Widal tube or the surface of a placenta from the pulp obtained by splenic puncture or from a smear of the bone marrow But, whatever may be the source of the blood the principles of diagnosis are the same—the parasites are sought in the red blood corpuscles, or their by products in the phagocytes The differences lie in the methods of approach This chapter reviews some of these methods excluding those which involve the delays of the normal histological procedures

DIAGNOSIS DURING LIFE

It is in the pure and fresh serum in the midst of the blood corpuscles that the parasites are best studied Laveran s statement has not stood the test of time In fresh blood the unpigmented organisms are vague and shadowy and easily overlooked and the stained film or smear has long supplanted the fresh preparation for routine diagnosis Yet we can ill afford to abandon the methods of Laveran Only by an examination of fresh blood can we study the movements of the parasites and the refinements of distinction in the patterns of the pigment which may sometimes provide the clue to the recognition of species and phase and an occasional examination of the living parasites can scarcely fail to bring a deeper meaning to the appearances we see in fixed and stained material Would it be fanciful to suggest that Marchiafava and Bignami described the parasites which were later called *Plasmodium tenue* when from a study of the living *P falciparum* they wrote of forms in motion which every moment are seen to take successively the strangest shapes and we may perhaps recall with humility that it was from the living parasites that Golgi first defined the unfolding of the erythrocytic cycle

Finger blood*—The routine diagnosis of malaria during life depends on the recognition of the parasites *in stained films of blood from the peripheral circulation* The source of the blood whether capillary venous or arterial is not important in diagnosis as the parasite density throughout the peripheral vascular system is fairly uniform but the capillary blood obtained by a needle prick of a finger or an ear lobe has obvious advantages *All other methods are secondary and appropriate only under rather special circumstances*

Bone marrow—Parasite forms which seldom reach the peripheral blood are common in the bone marrow The segmenting forms and the early gametocytes of *P falciparum* for example seldom appear in blood films though they are often found in marrow smears This observation first made by the early Italian workers has led to a belief that an examination of the bone marrow may sometimes reveal the presence of infection when parasites are not found in the peripheral blood and for many years malaria workers have been attracted by the diagnostic

* [illegible]

THAYER W S [illegible] H[illegible] K[illegible] Lond[illegible]

possibilities of the marrow smear But the published evidence on the relative values of blood and marrow preparations is curiously conflicting and we are left to presume that marrow smears have no assured place in the routine diagnosis of malaria though they be useful in special circumstances

Films or smears of bone marrow for malaria diagnosis are usually obtained by sternal puncture The technique is simple and apparently safe though rather frightening to sensitive patients The main advantage claimed is that parasites may sometimes be found by sternal biopsy in chronic or suppressed infections when repeated examinations of the blood are negative No such advantage is likely in acute infections Zacharias (1949) from an examination of parallel films from the blood and sternal marrow in 41 cases of acute falciparum malaria concluded that the density of parasites was slightly higher in the peripheral blood than in the sternal marrow and that sternal puncture is not a justifiable diagnostic procedure in acute falciparum infection In vivax malaria Knutgen (1949) found that the parasites in the bone marrow did not differ in numbers or distribution from those in the peripheral blood but that the continued presence of pigment in the macrophages after the attack provided a clue to the diagnosis Fischer (1949) states that sternal marrow examination does not help in diagnosis if the blood examination is negative and is thus valueless in diagnosis Aitken (1943) on the other hand in a careful study of 95 patients believed on clinical grounds to have malaria found that an examination of the sternal marrow was significantly more efficient than blood examination With one exception blood examinations were negative while sternal puncture yielded positive findings in 39 patients Rumball *et al* (1943) reaching similar conclusions state their opinion that sternal puncture has a special value in the diagnosis of latent malaria presenting as obscure illness and that in such cases it is a useful supplement to blood examination Bergeret (1951) has expressed a similar view

Aitken (1943) drew attention to the numerous artefacts in bone marrow films and smears Platelets often aberrant in form are more numerous than in the peripheral circulation and may lead to errors in diagnosis Howell Jolly bodies by no means a rare finding in the bone marrow may be mistaken for the chromatin of ring forms and basophilic red cells often large irregularly stained and stippled may present puzzling appearances resembling erythrocytes containing Schüffner s dots and badly stained trophozoites Portions of disintegrated myeloid cells may be interpreted as stages in the development of schizonts The non specific nature of these artefacts is usually recognised by the absence of malaria pigment and of a containing erythrocyte even so the interpretation of stained marrow preparations is not a responsibility to be undertaken lightly by the inexperienced

Spleen— The abstraction of blood from the spleen is unnecessary as well for diagnostic as for experimental purposes whilst for the first purpose the examination of blood from the finger is completely sufficient and for the second splenic blood gives very meagre results as compared with those obtained post mortem The operation is only indicated under exceptional circumstances (Mannaberg 1894) The segmenting forms of *P falciparum* may be found in

Z ch ar P J 94 The di gnostic alue f bone marrow b opsy mal ria caused by *Pl mod um f lc parum Am tr p M d I* 43 9

K t H 949 Knochenma k befund bei Mala ia ertiana *Z hr Tropenmed Par* I 8 Abs act *T en Di I ll* 95 47 99

F sch O 49) Ube F geb se vo S rnalpunk ionen bei Ma ia *Kl Woch* 27 235 A tract *Trop Di Bull* 95 47 309

Aitk C J 943 S rn l p nct the diagnos of mal ria *La* 496

R M ALL C A P sc s-Sm th B G a d Nancy d L (943 S ernal punct re in the d gnos of ma aria *Lan* 8

B ge et C 95) Not ur l d la her he des hema on res dans la moelle osseuse *Bul M d de l A rique Occid F* nc se 8 9 Ab act *Trop Di Bu* 1952 49)

M nn 1894 (ref p 5

the spleen when they are absent in the peripheral blood and spleen smears may reveal all stages in the phagocytosis of parasites and pigment and we may recall as a point of local historical interest that the pioneer studies of Wright (1901) on the malaria parasites of Malaya were aided by the examination of splenic smears But the potential risk of spleen puncture outweighs the possible advantages and most workers to day would probably accept the view of Mannaberg that splenic puncture has no place in malaria diagnosis

Placenta—With its slow blood circulation and large vascular spaces the placenta is a common site for great accumulations of parasites, particularly of the segmenting form of *P falciparum* Heavy placental infections may occur with few parasites in blood films and parasites are sometimes found in the placenta when they are apparently absent from the peripheral blood Clark and Tomlinson (1949) give data based on the observations of Walker and Wright in Sierra Leone on the relative value of the placental and blood films in diagnosis Parasites nearly always *P falciparum* were found in 199 of a series of 1 603 placentae examined in 88 women with heavy placental infections parasites were always found in the peripheral blood while blood examinations were negative in about one third of the remaining 111 women with light placental infection Blacklock and Gordon (19-5) record a similar finding among 150 parturient native women again in Sierra Leone the placenta was infected with *P falciparum* in 55 *of whom 45 showed no demonstrable infection in the peripheral blood*

The placenta is usually an effective barrier against the transmission of malaria infection from mother to child even when the placenta is heavily infected But the barrier may fail as in the congenital quartan infection so graphically described by Sir Thomas Browne in a letter written in or about the year 1679 to his son Sir Ralph Hare s sister fell into a genuine quartan when shee was yong with child which held her long and when shee came to her time shee was delivered of a daughter in a fitt and the child was so ill that none thought shee would live butt it grewe better but on the third day, about the hower of its birth fell into a fitt of an ague *and so agayne the third day after and that quartane settled and lasted for divers moneths* till shee was emaciated to skinne and bone but shee is now of the age of 18 yeares and a very full young *woeman* * Why the placenta normally so efficient in protecting the foetus from malaria infection should sometimes fail whether on account of injury during birth or from some other cause is not known

Most of the published information on congenital infection in malaria comes from *territories where the maternal infection rate and presumably the maternal immunity* is fairly high Congenital malaria under these circumstances is apparently rare in the series summarised in table 22 13 congenital infections only were recorded among 3 676 infections Jones (1950) however reporting 3 congenital infections among 54 infants born of European mothers makes the interesting suggestion that the efficiency of the placenta as a barrier to infection may be related to immunity in the mother Peel and Van Hoof (1948a) moreover have found that the placentae of native women in endemic areas contain an enormous number of phagocytes—a presumptive obstacle to the transplacental passage of the organism

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* Q ted by Moor T K (1907) *B m d J* 396

WRIGHT 90 (f p 3)

BLACKLOCK D B AND GORDON R M (1925) Malar inf cti n to cure n l t p gnancy ts l u hip t l bour nd arly nfancy *A n p M d Pa ass* 19 327

CLARK H C AND TOML NSON W J Th p th l g n t my f malaria Ch pter 37 in *Mal logy* Ed M F Boyd W B S und Lo d n and Phil d lph 1949

Table 22—MALARIA INFECTION IN THE MATERNAL, PLACENTAL, CORD, AND INFANT, BLOOD*

Authors	Cases	Percentage with parasites: Maternal blood	Placental blood	Cord blood	Infant blood
Clark (1915)	400	-	5	0 25	—
Blacklock and Gordon (1925)	155	6	38	0	0
Schwetz and Peel (1934)	56	68	74	6	4
Garnham (1938)	404	30	27	no congenital infections	
Peel and Van Hoof (1948a)	403	66	49		
Bruce Chwatt (1949)	450	—	6		
Walton (1947)	2 154	Seven congenital infections			
Jones (1950)	54	Three congenital infections			

* I f on mai ly due o P f l pa m

Skin—Peel and Van Hoof (1948b) have recently drawn attention to the possible and hitherto unsuspected rôle of the skin as a site for the schizogonic development of *P falciparum* From fifty infants and young children in the Belgian Congo known to have falciparum malaria they examined films which had been prepared from the tissue juice expressed after scarifying the skin In these films they found *P falciparum* at an advanced stage of growth sometimes as many as one mature schizont in each thin film field whereas ring forms only were present in blood films The parasite densities in the finger blood and in the tissue juice were about the same Their observations have been confirmed by Van den Berghe and Chardome (1951) who state that parasites may often be found by the skin scarification method when they are not seen in blood films Recently Chardome and Janssen (1952) have found the method to be efficient also in the diagnosis of vivax and malariae infections

These interesting and important observations were made among the indigenous peoples of the Belgian Congo where the malaria is hyperendemic The results of trials elsewhere particularly in areas where there is less exposure to repeated infection will be awaited with great interest

Venous blood—Malaria diagnosis from venous blood is appropriate only under special circumstances The blood is conveniently drawn from a vein for parasite cultures for concentration methods of examination and for the preparation of a large number of films as material for teaching

From blood clot diagnosis during life is often po sible but seldom necessary The method is appropriate when the blood clot alone is available for examination when for example blood has been sent to the laboratory in a test tube for some other examination and a search for

CLAR H C (9 5) Th d agnosti value f he placen al blood film ... o-a tumnal malaria *J p Med* 2 427
SCHWETZ J AND M PEEL (934 Co g nital malaria and place al infections mong t the negroes of Ce al Af ica. *T R Soc rop M d Hyg* 25 67
GARNHAM P C. C. 938) The place in mala ia wi h pecial ref rence t et cul -endothelial immunity *T an R Soc p M d Hyg* 3 3
PEEL, L n V n Hoof L. (948 Le palud me à la ma er té ind gène de Léopold il. *Ann Soc B lg d M d trop* 8 4) (Ab act *Trop D Bull* 1949, 46 699)
WALTO , G A (4) On he con rol of mal ia in Freet wn, Sierra Leone 1 *Pl smodium f l parum* d *Anophe g mbiae* relat on malaria occurring i fan *Ann rop M d P as* 41 38
JON s, B S 95) Congeni l mala ia R port of three cases *Bri med J* 439
B UCE-CHW TT 949 C d by Jones (95
PE L, F nd V H oF L (948b) Comportemen d *Pl smodium f l parum* dans le derme hez l'enfan ind g ne *A Soc B g d M d Trop* 8 273 (Abs act *Trop D Bull* 949 4 7
V N DEN BE GH L CH DOM M (1951 An asier nd mor accura diagnosis of mala ia and filariasi hrough the use f the k scarification me *Amer J trop M d* 31 4 1
CHARDOM M AND JANSSEN P J (95 Enqu ur l incidence malarienne pa la m hode dermique dans la r g on d Lubumh (Congo Be ge) 4 *Soc B g de M d trop* 3 09 (Abstract *Trop D Bull* 95 49 095

malaria also is thought necessary For a short time after the blood is drawn the parasites show little change in appearance Within a few hours however, they begin to contract and to stain so deeply that the distinction between cytoplasm and chromatin is lost and the organisms resemble large cocci and after about twenty four hours with the clot kept at the temperature of tropical countries, the parasites become degenerate and unrecognisable

Malaria diagnosis from clotted venous blood is conveniently made from thin or thick films The films may be taken direct from the clot but films prepared in this way are likely to be rather messy and the appearances of the parasites may be confusing A more laborious but better method has been suggested by Denes (1931) The serum having been removed with a pipette and the clot shaken up and then discarded the dregs are spun in a centrifuge and films are prepared from the deposit

Pigmented leucocytes— There are cases of mild summer tertian where sometimes it is impossible to find parasites in the blood of the finger during short intervals of time, but the pigmented white blood corpuscles enable the physician to diagnose the infection as malarial. Even after a cure has been effected by the extinction of the acute infection one may still see pigmented leucocytes in the blood for five six, or eight days The polynucleated cells are not so often pigmented as the large mononucleated cells, while the lymphocytes and eosinophil white blood corpuscles never contain pigment or other debris (Marchiafava and Bignami 1894) We can add little to these observations Phagocytosis of parasites and pigment by the large mononuclear leucocytes and by the neutrophil polymorphs, occurs throughout the reticulo endothelial and vascular systems, particularly in certain of the viscera The phenomenon is most active soon after segmentation it may be seen in films from the peripheral blood though only as a minor episode in a drama which is played out mainly in the inner organs The pigment bearing leucocytes circulate for some days even when the parasites have disappeared from the circulation as a result of treatment When seen in blood films they are conclusive evidence of present or past malaria the only decisive microscopic evidence apart from the actual finding of the parasites

Leucocytes containing malaria pigment are often seen in blood films during acute attacks more often in falciparum than in vivax or quartan malaria and in heavy rather than in light infections They seldom contribute to diagnosis during acute attacks but on occasion during an ebb in parasitaemia or for a few days after the subsidence of an attack they may bring positive evidence of infection when the search for parasites has been unsuccessful

Blood picture—Marchiafava and Bignami (1894) held the view that in most cases the white corpuscles decrease in number both relatively and absolutely during the malarial infection so that when the blood of persons suffering from malaria happens to show a considerable increase of polynucleated neutrophil white blood corpuscles we must assume the existence of another infection Some years later Thomson (1911) working under the direction *of Ross formulated three laws governing the behaviour of the leucocytes in malaria*

Law 1—During active malaria the number of total leucocytes in the peripheral blood is below normal and varies more or less inversely with the temperature

Law 2—In malarial fever the curve representing the percentage of total mononuclear leucocytes is the exact inverse of the temperature curve

Law 3—In convalescent or apparently cured malaria transient periodic leucocytoses occur in the peripheral blood and these leucocyte fluctuations arise chiefly from a polynuclear variation

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DENE G (1931) R h h du p te malariq e d na l a g v neux. (*Ab te ct T op Di Bull* 1932 29 355)
THOMSON D (19) Th l ucocyt in mal ial f m thod f d gn og m laria l g f t is pp ently cur d. *An p M d l* ar 5 83

The rhythmic swing in the number of mononuclear cells over an amplitude of from 20 to 40 per cent continues into convalescence and according to Thomson justifies a diagnosis of malaria even though parasites are no longer found in the blood (The term mononuclears is used by Thomson to include lymphocytes as well as the cells now more usually known as large mononuclears)

Something of the dignity of these laws has been lost with the years They remain broadly valid but they have been weakened by inconsistency and they are no longer held as a basis for confident malaria diagnosis Kop (1921) for example found the mononuclear increase in malaria to be capricious and suggested that a shift to the left in the Arneth count—that is a fall in the number of lobes in the polymorphs—might be a more constant diagnostic sign Manceaux (1939) states that early in the attack there may be a leucocytosis which gives place to a leucopenia as the attack progresses James (1922) refers to an early leucocytosis particularly in falciparum infections while Kitchen (1949) found little variation in the total leucocyte count during attacks With these inconsistencies it seems that little reliance can be placed on leucocyte counts in the diagnosis of malaria but that certain changes should arouse a suspicion of malaria and encourage a sustained search for the parasites These changes are—(*a*) a leucopenia with a relative mononucleosis in fevers of unknown origin and (*b*) a rhythmic fluctuation on the mononuclear counts in suspected chronic malaria

Provocative methods of diagnosis—The difficulties of diagnosis in latent malaria have long been known The parasites may be absent from the blood circulation and a suspicion of malaria arising from a suggestive history and an enlarged spleen may be hard to confirm There is evidence however that under appropriate physical or chemical stimuli parasites may be washed into the blood stream from their hidden retreats in the internal capillaries and numerous attempts have been made to turn this effect to diagnostic advantage the so-called provocative methods of diagnosis

Sassen (1919) and Padilla (1926) have classified the provocative agents in malaria

1 substances acting through protein shock—serum vaccines milk
2 substances acting on the circulation—ergot adrenalin pituitary extract strychnine
3 substances acting on the parasites—quinine arsenic
4 physical agents—hot or cold douches or packs vapour baths massage and faradization of the spleen X rays insolation and ultra violet rays (fatigue and exposure to cold should perhaps be added to this list)

Numerous reports suggest that exposure to one or other of these stimuli may provoke a relapse in malaria but the results of a deliberate application of provocative methods for malaria diagnosis have been inconsistent and their value has not been generally accepted. The best methods according to Sassen (1919) are those which depend on injections of adrenalin or exposure to light rays with physical exercise Dazzi (1919) and Boujoucos (1933) believe that

Kop W A 1921 The change in the leucocytic blood picture as a symptom of malaria. *J trop Med (Hyg)* 24 218

Manceaux A 1939 La formule leucocytaire dans le paludisme aigu. *Bull Soc Path exot* 32 15

James S P Chapter in *The Practice of Medicine in the Tropics* Ed W Byam and R G Archibald Henry Frowde and Hodder and Stoughton, London, 1922

Kitchen S F Chapter 4 in *Malariology* Ed M F Boyd W B Saunders Co London and Philadelphia, 1949

Sassen P 1919 Über die Methode der Malariaprovokation *Arch Schiffs u Tropenhyg* 23 35 (Abstract *Trop Dis Bull* 1919 14 7)

Padilla T 1926 Pruebas diagnosticas del paludismo latente (Abstract *Trop Dis Bull* 1927 24 258)

Dazzi A 1919 Le iniezioni di adrenalina per la diagnosi di malaria latente (Abstract *Trop Dis Bull* 1920 15 401)

Boujoucos B 1933 La mobilisation des hématozoaires vers la circulation périphérique sous l'influence de l'adrénaline *Bull Soc Path exot* 26 594

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DENES G (1931) R h h du p mal nq dans l ang v in ux (Ab tr ct T op Di B ll 1932 29 355)
TH MSON D (191) The l u ocyt in mala l f a m th d f d gn ing mal ria lo g f t is ppar tly cur d Ann t p M d P as 5 83

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KOP W A (9) Th hang in th l cocytic blood p tur ymp m f m l *J p M d (Hyg) 24* 78

MANCE UX, A (939) La f m l l ucocytair d l p l di m agu *B ll S P* *3* 5

JAMES S P Ch p 7 in *Th Pr f M d cine he T p cs* Ed W By m d R G Ar hib ld H nry F wd d Hodd d Sto gh on Lo d 9

KI CHEN S F Chapte 4 *M l ri l gy* Ed M F B yd W B S d rs Co London d Phil d lphia 949

SASSEN P (9 9) U b di M th de d Malariap ov k *A h S h ff T p hyg 3* 35 (Ab ct *T p Di B ll* 9 9 *14* 7)

PADILLA, T (9 6) Pru b diagn cas d l pal di m la te (Ab tract *T p D B ll* 9 7 *4* 58)

DAZZI A (9 9) Le ini oni di dr alina pe la diagnos di malaria l (Ab tra *T op Di B ll* 9 *15* 4)

B Y cos B (933) La m bili des h m t ai es rs la circul tion pé ph riq l nfluen d l d é l *B ll Soc P xo 6* 594

adrenalin is the most useful drug they state that a subcutaneous injection of one milligram drives the parasites into the blood stream and does not provoke an attack the parasites remain in the blood for several hours, diagnosis is usually possible after recent attacks but often fails during prolonged latency Sinton (1925), too found that adrenalin injections had little diagnostic value during latency in vivax malaria

The behaviour of the malaria parasites during latency is now better known vivax infections may persist in the parenchymal cells of the liver rather than in the sinuses of the spleen and under these circumstances adrenalin injections would be likely to fail When however there is a lingering low grade erythrocytic infection an injection of adrenalin may have a limited diagnostic value

Cultivation of the parasites—The growth of malaria parasites normally ceases in blood which has been withdrawn from the body Bass and Johns were the first to show that continued growth in a test tube for at least one generation is possible when a little dextrose is added to blood from which the fibrin has been removed The method is simple, and the use of parasite cultures as an aid to diagnosis has been suggested on the grounds that parasites may sometimes be recognised in cultures when ordinary blood films are negative Parasites advanced in growth it was thought, might be more easily recognised than young rings the selective layering of infected cells might lead to a concentration of the parasites and there seemed also to be a possibility that the organisms might actually multiply in culture Knowles and Senior White (1927) regarded the cultivation of the parasites as one of the best methods of malaria diagnosis in difficult and obscure cases Field (1932) on the other hand in a comparison of parasite cultures and thick blood films in 76 acute infections found that cultures contributed little which could not be got more simply from ordinary blood films Diagnosis from cultures it seems might be useful when the parasites are few Our own experience however leads us to believe that the time taken to cultivate the organisms might be spent more usefully in a thorough examination of serial thick films

Concentration of the parasites—The first attempt to concentrate malaria parasites for quicker diagnosis seems to have been made by Laveran (1891) who added a drop of water to lake the blood and was able in this way to throw the crescents into bolder relief There was little concentration of the organisms but the germ of the idea was there Ten years later Ross (1903) adopting the same principle of haemolysis obtained the first effective concentration in the thick film method which bears his name This method modified and improved is now a standard method of malaria diagnosis throughout the world

The concentration of parasites attained by the thick film method has physical limitations Excessively thick films are not transparent enough for microscopic examination and a twenty to forty fold concentration is the useful limit Further concentration is possible however if one takes advantage of the difference in specific gravity between infected and non infected cells The red cells containing the larger parasites are lighter than normal cells they tend to rise to the top when the blood is prevented from clotting and allowed to settle or else spun in a centrifuge The leucocytes are still lighter The column of cells in the spun tube will hence separate into layers with the leucocytes at the top the infected erythrocytes just beneath and

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SINTON J A (1925) St d n mal ria with p cal f n t tr m nt III Th al e f provocative method in th d gn f uncu d malarial inf ti n *Ind n J n d R* *13* 603

BASS AND JOHNS 1912 (f p 33)

KNOWLES R AND SENIOR WHITE R *M l ri* *m g* *nd* *l w k p al f* *I d n nd n* Th ker Spink and Company Calcutta 1927

FIELD 1932 (f p 3)

LAVERAN 1891 (f p 2)

ROSS R (1903) A mp d method f the m sc p al d gn f int rmitt t f *Lanc* 86

the uninfected cells at the bottom Bass and Johns (1915) in the course of their work on the cultivation of the parasites were apparently the first to recognise this sepa ation of the infected cells in blood suitably treated and to devise a practical and effective method of concentrating the larger parasites without destroying the red cells Their method described on p 109 achieved a high concentration of the larger parasites failing only with *P malariae* and the young tropho zoites of *P falciparum* The principles laid down by Bass and Johns have been applied by later workers with many variations from the simple method of Landeiro (1934) who used the sedimented blood in a Westergren tube to the elaborate method of Berliner *et al* (1948) who obtained a concentration of *P vivax* so high that infection could be recognised when the density in the original blood was as low as one parasite for every ten cubic millimeters For a description of some of these methods the interested reader is referred to the original papers Their main defect is that they are efficient only with *P vivax* and with the gametocytes of *P fal iparum*

Other workers have sought to concentrate the parasites by laking the blood and preparing films from the centrifuged deposit One of the early methods was that of Krauss and Fleming (1916) who added the blood to a citrate saline formalin solution and prepared films from the final deposit after the blood had been spun twice in a centrifuge—the first time to separate the leucocytes the second to throw down the parasites Halawani and Sobky (1939) recommended the addition of 0 5 c cm of blood to 10 c cm of a one per cent acetic acid solution the mixture was spun for 10 minutes at high speed and films were made from the deposit Szauter (1937) devised a method based on the same principles Christophers and Fulton (1939) working with *P knowlesi* obtained a cleaner lysis of the blood by mixing the sedimented cells with an equal volume of saline and adding 0 2 per cent saponin to the mixture In all these methods the red cells are destroyed but unlike the methods which depend on a layering of cells of differing specific gravity these methods concentrate all the parasites including the young rings of *P falciparum* Their main drawback is that the parasites are sometimes distorted and difficult to recognise

Concentration methods take time and they depend on the resources of a well equipped laboratory They are necessary when a high density of parasites is needed for investigation and they have a limited clinical value under appropriate conditions but our own experience of several of the methods described has left us with an unshaken confidence in the ordinary thick film methods for routine diagnosis

DIAGNOSIS AFTER DEATH

Heinrich Meckel (1847) seems to have been the first worker to find and describe pigmented bodies—which were almost certainly malaria parasites—in the blood and organs of a patient who had died from malaria The subject a patient in a lunatic asylum had suffered from malaria and post mortem examination revealed a very large spleen and a slate coloured staining of the grey

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B C C N J NS F M (9 5) A method f tı g mal plasmodı f d gnos d h p pos *A er J p Dı 3* 98

LAN F (934) Anr h gsm th d fü d U h g d Mal p as m Bl *A h S h ff T p hyg 38* 53 (Ab tr *T p Dı B ll* 935 *3* 788)

BERL NE R W K NNED T J J AN B GELOW F S (948) A t hnıque f h d t f mı mal umb f mal n p es ppl ın h d ct on f pp d vı m lan *J l I* 27 N 3 P 34

R RS K B (946) Conce tr ting mal p as ın hı film *Brı m d J*

ARU L (948) L d l p as malar m d ar h m *R M l n l 27* 47 (Ab tr t *T p Dı B ll* 948 *45* 97)

KRA W ND FLEM N J S (9 6) A tr tı m hod f the dı g os f mal *S her m d J 9* 141 (Ab tr t *T p Dı B ll* 9 6 *7* 69)

HAL WANI A AND SOB M F (939) O th ffi cy f th me hod f nc t f bl od p t th diagn f m l ria (Ab tr *T op D Bull* 94 *37* 864)

S UT B (937) N V rf hr (D chtu gs rfahr) ur D gn d pı h Malar *Z l B k* Abt Orig *138* 3 3 (Ab tract *T p D B ll* 937 *34* 590)

CHR PH S R ND FUL N J D (939) E p rım with l ted mala par (*Pl m d k owlesi*) f ee f m d cell *A p M d P 33* 6

MECK H (847) U b hw rz P gm ınd Mıl d m Bl eın G teskr nk *Z h f P y h n S* 198 (Cı d by Ma nab g 893 f p 5)

substance of the brain The blood from all parts of the body contained black pigment either lying in the capillaries or squeezed out of the vessels Invariably a more or less great number of black irregular granules were united by a colourless substance to a globular, *egg shaped or* fusiform body, the size of these bodies amounted to 002 to 007 of a line * The bodies were most numerous in the vessels of the grey matter of the brain We may surmise that these bodies were the schizogonic forms or gametocytes of *P falciparum* and in this description we *may perhaps recognise the beginnings of autopsy* diagnosis in malaria based on the presence of the organisms But more than thirty years were to pass before the pigmented bodies were seen in their true light as living organisms

Deaths from acute malaria are nearly always due to *Plasmodium falciparum* Few patients die from uncomplicated vivax or quartan malaria though repeated attacks in longstanding infections may contribute to a fatal issue The Malaria Research Division of this Institute has treated 1,362 patients for acute vivax and malariae infections in past years without a death directly attributable *to the malaria, but 57 of the 2 450 patients treated for acute falciparum* malaria died as a direct result of the infection Clark and Tomlinson (1949) report a similar difference in Panama, in a study based on autopsy material from 405 patients who had died from malaria *P falciparum* was the only parasite involved We may perhaps deduce from this evidence that deaths attributable directly to malaria are likely to be due to *P falciparum*, and that the discovery at autopsy of small numbers of P *vivax P malariae* or *P ovale* does not necessarily mean that parasites of these species were the cause of death

Positive evidence of malaria from autopsy examination must come from the microscopic demonstration of malaria parasites or pigment in sections smears or films of the tissues The refinements of histological examination desirable though they may be are often denied to workers in the under developed areas of tropical countries and in this account we shall consider *only those methods of examination which are practical in any small up-country hospital* or dispensary

AUTOPSY WITHOUT DISSECTION

In Muslim countries, there are strong traditional and religious objections to post mortem examinations which involve dissection of the body In these circumstances the diagnosis of malaria after death must be made from blood taken from the superficial capillaries or veins or from material withdrawn from the body with a syringe and a large exploring needle It will seldom be possible to get satisfactory films from the finger blood—the blood *in the superficial* capillaries soon becomes clotted or laked—but for some hours it may be possible to obtain blood from the superficial veins Some of the films from which Watson (1903) make his pioneer observations on the development of the crescent were taken two and a half hours after death from the superficial veins of the arms Later the tissues most likely to yield positive evidence are the brain the heart blood, and the spleen

Brain tissues sufficient for malaria diagnosis may be obtained by the method recommended *by Daniels and Newham (1923) A large exploring needle* is passed through the orbital plate of the frontal bone suction applied with a syringe after stirring the brain a little with the needle will usually bring away enough tissue for examination There is no disfigurement as the needle is passed through the conjunctiva under cover of the eyelid The brain tissue while still moist is *squeezed between two slides which are quickly* separated in the plane of the surfaces to produce two thin smears The colour drawing which appears as plate XLVI was made from a brain

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* A li p um bly millim tr

Clark and Toml n on 1949 (f p 84)

Watson M (1903) A not n th p f ca e f mal gn t mal ia with discus n th d lopm t f th cres nt *J t op M d (Hyg)* 6 2

Dan els C W and Newham H B Labor t ry Stud in T p cal M dicin J hn B l Son and Dan l on Ltd. London 9 3

smear prepared by Dr J F B Edeson in this way Fuller details of the preparation and staining of the smears are given on p 105

Diagnosis may also be attempted from the heart blood or spleen pulp similarly obtained with an exploring needle The blood in the cavities of the heart remains fluid for a variable time after death and the spleen pulp is not seriously affected by post mortem changes for a few hours With prolonged delay however the heart blood clots and becomes crowded with saprophytic organisms while the parasites and cellular elements of the spleen may become so degenerate as to be unrecognisable in stained preparations The methods of preparing films and smears are more fully discussed on pp 102 107

AUTOPSY WITH DISSECTION

When death is due to acute malaria the parasites are likely to be distributed throughout the vascular organs The density of parasites will probably be high though death may occur when the numbers have been greatly reduced by treatment (*see* table 5) Positive evidence of malaria will usually depend on the demonstration of parasites or pigment in the brain the blood of the heart or great veins the spleen pulp or the bone marrow * Kean and Smith (1944) have recorded the frequency with which parasites were found at autopsy in these tissues (table 23)

Table 23—MALARIA PARASITES OR PIGMENT IN VARIOUS ORGANS FOUND AT AUTOPSY IN ONE HUNDRED FATAL CASES OF FALCIPARUM MALARIA (data from Kean and Smith 1944)

	Blood	Brain	Spleen	Bone marrow
Parasites	66/96	71/100	68/98	58/87
Plugging of capillaries	—	16/97	—	—
Pigment	—	68/100	91/99	64/87

Blood—Blood for the preparation of films may be got from the heart or from the portal vein The blood may not clot for some considerable time and the parasites may be easily identified in films made within a few hours of death But the difficulties increase with delay and after about twenty four hours diagnosis becomes unreliable the films are likely to be poor and the parasites unrecognisable while a rank growth of saprophytic bacteria may obscure the microscopic picture

Though the parasites in blood taken from the dead body may be readily identified they are often changed in appearance For a time the young trophozoites retain the usual ring form but after a few hours they tend to contract and to appear in stained films as small solid looking bodies superficially resembling large cocci

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Wh th esource f w ll q pp d lab ra ry ailabl thi nf m will us lly b ppl me d by hi tol gical mi f th gan Small p ce f b q art f h thi k h uld b pl d oon h y cu lab lled tub taini g b tw ty tim th l m f bl fixati Th g f ti might in l d ce bral rt nd basal l pl l h rt muscl d nal lung and m ll in tin F rmal-s h us ful mpl fixati (pe ce f m lin mal l wh h f w hips f cal m carbon l m h b add d eu ral the f rm d lea d) F mal fi ti whi h d in acti p od on malarial p gm f mald hyd p gm d f mald hyd h mati wh h y ti ri h blood Thi p gm m y mbl mal pigme nd f d gnos D A T H Marsde P th l gi t f hi I tu d f m thi sour by g b ff d f malin fixa w th h f rmula

F rmali (37 pe t f rmald hyd l ti)	oo
W	900
A d sodi m phosph m hyd	4 gm
Anhyd us disodi m phosph	6 5 gm

A th good fixa Carnoy fluid wh h th p ri f on f (P G S) b tha f mal and m b d f ti whi h ar b ai d w th G ms ai Car y fluid ha h dr wb k how ha p od ly f th d ll The f rm l

Gl cial cid	p rt
Ab l l h l	6 par
Chlor f m	3 parts

K AN B H AND SM H J A (944) D th d t es o- mnal mal ri A umé f hund d psy 9 5 94 *Amer J tr p M d* 24 3 7

Brain—When the brain is hea ily infected the parasites can usually be demonstrated in the capillaries for many hours after death, either in fresh material or in stained smears

Fresh brain may be examined under a coverslip A fragment of cortex or medulla is placed on a glass slide and squeezed thin beneath a coverslip until it spreads to the edge When the parasites are advanced in growth the pigment granules ranged in long lines along the course of the capillaries may be recognised with a 1/6 dry objective, and readily identified with the 1/12" oil immersion The use of polarised light is a considerable help in the examination of the unstained material (p 94) The distribution of infection in the brain is sometimes patchy, and material from several areas should be examined including the basal nuclei and the cerebellum

Excellent brain smears may be prepared by the squash' method described on p 105 The capillaries are well preserved and can often be traced for a longer distance than is possible in sections The parasites should be sought within the capillaries, though a few may be found lying fre They are usually advanced in growth, often with well formed pigment, as it is at this stage that stagnation in the brain seems to occur (plate XVII) Their numbers vary from very few to massive accumulations completely filling the distended capillaries Phagocytic cells heavily laden with malaria pigment and resembling those found in the peripheral blood may aid diagnosis when the parasites are few but phagocytosis is much less evident in the brain than in the spleen and bone marrow For some hours after death, even at the temperatures of tropical countries the parasites are likely to stain well enough to be recognised but after about twenty four hours the cytopla m disintegrates and the stained chromatin has a blue tinge In these circumstances the characteristic pigment may provide the clue to diagnosis

When the cerebral capillaries are but lightly infected we may presume that the parasites during life were able to circulate freely and that the brain was probably no more involved in the infection than other organs But when a patient dies in coma and the brain capillaries are packed tight with parasites the assumption is not unreasonable that the two phenomena are related Marchiafava and Bignami (1894) in their study of the malignant malaria fevers, give a series of illuminating clinical descriptions of severe falciparum malaria, with illustrative cases Among their patients were five who died in coma four with massive brain infection * This early reco d illustrates a relationship which has since been generally accepted, though not without reservation Their fifth patient was admitted to hospital in coma with an immense quantity of plasmodia in the blood He remained in coma for two and a half days and then he died despite energetic quinine treatment The brain was lightly infected only a few capillaries containing parasites Coma it seems, developed in this patient without the spectacular blocking of the brain capillaries found in the other four cases

These observations have been amply confirmed Stupor coma and the other central nervous symptoms of cerebral malaria, are often associated with heavy accumulations of parasites in the brain but there are cases in which this apparent causal relationship is les clear Kean and Smith (1944), for example in their studies on fatal falciparum infection record twelve cases which though classified on clinical grounds as cerebral malaria, showed no particular accumulation of parasites in the brain For this apparent anomaly there seems to be no complete explanation The main assumptions open to us are

(a) that a blockage of the brain capillaries always occurs in cerebral malaria but that it may be transient and relieved by treatment even though the patient succumbs to the infection

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* These [illegible] symptoms with their detailed correlation of the course of the infection with the numbers and the stage of development of the pa[illegible] in the blood during life and in the organs after death have never been surpassed They might be re[illegible] with profit today by all students of malaria

MARCHIAFAVA AND BIGNAMI 1894 (ref p 3)

KEAN AND SMITH 1944 (ref p 91)

(*b*) that a focal blockage of cerebral capillaries may occur in areas not selected for examination at autopsy Clark and Tomlinson (1949) state that one area of the cortex may show only scattered parasites while other areas reveal almost complete infection of the red cells

(*c*) that stupor or coma in malaria may be due in some cases to blockage of the brain capillaries but that there are other causes

(*d*) that there is no causal relationship between the blocking of the brain capillaries and the cerebral symptoms of malaria

For fuller information on this important and essentially unresolved problem the reader is referred to the recent accounts of Maegraith (1948) Clark and Tomlinson (1949) Rigdon (1942) and to the studies of Knisely *et al* (1945) on the phenomenon of sludging of the blood in fatal knowlesi malaria in monkeys A short review of the problem with particular reference to sludging of the blood and to the theoretical promise of heparin in treatment will be found in a recent number of the Medical Journal of Australia

Spleen—Malaria parasites at all stages of schizogony young and mature gametocytes and large accumulations of malaria pigment in the phagocytes are often present in autopsy films or smears from the splenic pulp of patients who have died from falciparum malaria The density of parasites depends largely on the duration of treatment before death when the infections have been little influenced by treatment many parasites often mature segmenters may be found in every field of the microscope but a few days of treatment may greatly reduce their numbers Malaria pigment is always prominent particularly in the later stages of infection and in treated infections the pigment may afford the main perhaps the only diagnostic evidence The pigment may be scattered in small compact rounded masses derived from the mature segmenters but most of it is likely to be found aggregated into dense collections in the phagocytic cells

The parasites in the spleen pulp like those in the blood degenerate after the death of the host they undergo cytolysis stain badly and become difficult to identify These changes proceed rapidly in warm countries where degenerative changes in the parasites and cellular elements of the spleen may be far advanced within twenty four hours

Bone marrow—Autopsy smears from the bone marrow like the smears from the spleen pulp show parasites at all stages sometimes with young gametocytes in large numbers Malaria pigment may usually be seen both in the phagocytic cells and lying free but pigmentation is usually less than in spleen smears As in the spleen the number of parasites is likely to be related to the terminal parasitaemia after prolonged treatment they will probably be few in number or even absent

SPECIAL METHODS OF MICROSCOPY

Special optical effects designed to bring malaria parasites or particular structures within the parasites into clearer definition have been recommended The more important are those which depend on dark ground illumination fluorescence polarised light or light filters transmitting a particular band of the spectrum

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CLARK ND T M INSON 949 (r f p 84)

MA RA TH B G *P h l g l P* *Mal n d Bl kw er Fever* Bl kw ll S fi Publ O f d 948

R GD N R H (942) A d t f h m b m m f d th n cu *Pl d m f l p rum* inf tio po t f se *Amer J H g 36* 69

K SEL M H S RA MAN TH MAS W K E T S ND B OCH E H (945) K w l m l n m k ys I Microcop p h l g al ci cul ry phy l gy f h us m nk y dun g cu *Pl mod m knowl si* mal (A m tion p ctur) *J M l Soc 4* 85

M d l J A l h Jun 955 p 885

Dark ground microscopy—The use of dark field illumination in the examination of blood films for malaria is recommended by Wolter (1932) Goosmann (1936) and Packer (1945) The one outstanding merit of this method is the brilliant definition of malaria pigment. Parasites which contain pigment particularly gametocytes and advanced schizogonic forms stand out in brilliant contrast on the darkened field The method is said to be particularly suitable for demonstrating exflagellation of the male gametocytes The technique is less sensitive, however than the ordinary thick film technique and as the young unpigmented trophozoites are not visible in the darkfield the method is useless for routine diagnosis

Fluorescent microscopy—Malaria parasites fluoresce a bright yellow when stained in ordinary thin films with a weak solution of berberine sulphate or rivanol and examined by ultra violet light Unlike dark field microscopy which illumines only the pigment, the fluorescence method lights up all the parasites, even minute unpigmented rings Metcalf (1945) has used this method with success in routine diagnosis It is simple and apparently reliable but it needs special equipment and it involves a sacrifice of the stippling of the host red cells and of the colour contrasts within the parasites which are so useful for diagnosis in Romanowsky stained films A limited trial at this Institute by Dr J P F Whelan did not encourage us to proceed further with the method

Polarised light—Viewed by polarised light malaria pigment glows brightly on a dark field Most of the substances which might be mistaken for malaria pigment do not glow in this manner This behaviour, noted by Schaudinn many years ago, may sometimes be turned to diagnostic advantage Not only may the pigment distribution in a stained parasite be defined more clearly than is possible with ordinary light but malaria pigment may often be distinguished from pigment granules of non malarial origin The technique is extremely simple requiring no special equipment beyond a couple of pieces of Polaroid and a bright source of light With an ordinary stained film under the microscope and the light set as brightly as possible, one piece of Polaroid is placed in front of the microscope lamp replacing the filter, while the other is held in the hand and rotated above the eyepiece until the microscopic field becomes dark We have used this method for some years not for routine diagnosis—for which it is quite unnecessary—but as a check on the identity and distribution of pigment granules

Light filters—The colours of stained material can usually be enhanced by the use of suitable light filters The use of pale blue filters to reduce the excess of yellow rays in artificial light is normal practice in microscopy Cares (1943) recommends the use of a light red filter (Wratten light red filter E series 23A Eastman Kodak Company) to facilitate the recognition of the parasites in routine diagnosis This filter has a spectral transmission band parallel to that of eosin the red cells hence appear lighter while the blue stained cytoplasm appears darker due to the absorption of the blue and green The simplest form of the E filter is the dye stained gelatin film supplied in one inch and two inch squares, these can be cut and fitted either in front of the lamp above the sub stage condenser or in the eye piece We have tried this method but have not thought the advantages enough to compensate for the degradation in the colours of the Romanowsky stains Other special effects are useful mainly for photomicrography Deep blue filters are useful to darken the pigment and to throw the granules into sharp contrast while green filters give excellent definition of cytoplasm without unduly masking the chromatin

WOLTER A (93) Ueb di S hn lldi gno d Mal mit Hilf des Dunk lf ld (Ab tr *T p Di B ll* 932 *29* *355*)

GOOSMANN C (936) D k fi ld illuminati n in th di g f tub culos and mal ri *J Lab I Med 21* 42

PACKER H (945) Th of darkfi ld illumin ti n n studies f malari par e *J M l S* *4* 33

METCALF R L (1945) Th d t ti n f the pla modi f hum n mal ria in blood film by fl es mi os opy *J na M l S* *4* 3

CARES R (1943) Rap d di gn s f m laria by the e of a W at n light filt *J Lab I Med* *8* 750

CHAPTER VIII

TECHNICAL MISCELLANEA *

THE ROMANOWSKY STAINS

However obscure may be the mechanism of Romanowsky staining the empirical principles are simple The Romanowsky stains are neutral compounds in which both basic and acidic radicles have staining properties The basic stains are methylene blue and the oxidation products of methylene blue known as methylene azure the acid stain is eosin Combined these substances form a mixture of the eosinates of methylene blue and of methylene azure Methylene blue stains the cytoplasm of malaria parasites eosin stains the red cells eosin with azure stains the nuclear chromatin Ionic dissociation of the staining radicles occurs when water is added to alcoholic solutions of the stain dissociation at a pH around neutrality ensures the correct range of colour The permutations of technical method by which those requirements can be met are many and varied and the literature of the past sixty years bears witness to the incessant search for the ideal combination This account describes the particular methods by which the material for this Study was prepared and stained

The Romanowsky effect—Methylene blue is a poor stain for the nuclear material of malaria parasites so too is eosin But when the two stains are used together their action is modified if the methylene blue is old The nuclear chromatin then stains red This effect the Romanowsky effect has been explained by the assumption that the oxidation products of methylene blue act as a mordant preparing the chromatin for the staining action of the eosin But there are other explanations What is certain is that the effect is produced by a dual action of eosin and of the substances in methylene blue which have been called the red out of methylene blue To these substances the name *azures* has been given

The azures are oxidation products of methylene blue formed by the replacement of the CH groups of the molecule by H atoms Methylene blue is tetra methyl thionin The important azures are di methyl thionin also known as azure A and tri methyl thionin or azure B Stains which are rich in azure A are thought to have a special value in the staining of blood and bacteria for the staining of malaria parasites the more important substance is probably azure B

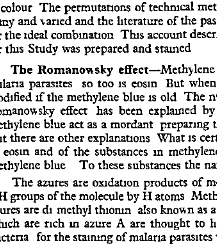


Fig 28—METHYLENE BLUE IS TETRA METHYL THIONIN CHLORIDE WHICH IS POLYCHROMED BY OXIDATION TO YIELD AZURES

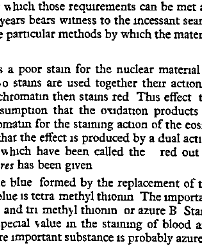


Fig 29—TRI METHYL THIONIN OR AZURE B IS PROBABLY AN ACTIVE AGENT IN THE ROMANOWSKY STAINING OF THE NUCLEAR CHROMATIN OF MALARIA PARASITES

(Lillie 1949) The azures are formed when methylene blue is exposed to alkalis acids or ultraviolet light The term *polychrome methylene blue* is often used for methylene blue which has been changed in this way

* T chnical me hod pp pria f malaria d gnos f m th k blood film ar mu ted f m thi b p having b n d cr b d in th li lum (S udy N 3 *Ins M d R* F d f Mal y)
Lil ie R D (949) A G ms tain *Amer J op M d* 29 6 5

The most widely used stains for malaria diagnosis from thin blood films and tissue smears are the particular combinations of polychrome methylene blue and eosin introduced by Giemsa and by Leishman Wright s stain, widely used in America is essentially the same as Leishman s, differing only in the method of polychroming These stains are essentially mixed eosinates of methylene blue and of various azures They are used as solutions in methyl alcohol or methyl alcohol and glycerine

Romanowsky staining and pH—Dissolved in methyl alcohol or in methyl alcohol and glycerine, the Romanowsky stains are effective only when they are diluted with water With the addition of water there is an ionic dissociation and the dissociated radicles of methylene blue, azure and eosin are adsorbed to or combine with particular tissue elements to give the distintive Romanowsky range of colour A highly important factor in the staining process is the pH at which dissociation takes place The best staining for malaria diagnosis is attained when the pH of the stain diluent lies between 7 0 and 7 2, any significant departure from this pH is likely to disturb the balance of colour Distilled water is usually acid from dissolved carbon dioxide, exposed to air in the laboratory it soon becomes useless for Romanowsky staining unless the acidity is neutralised A common method of adjusting the pH of the stain diluent is to add a few drops of dilute alkali solution Shute s method of staining thin films described on p 101, makes use of lithium carbonate to neutralise the stain A second method of controlling pH is to substitute a buffer solution for distilled water as a diluent for the stain a method which has been used for many years in this Institute

Estimation and correction of pH—Some means of determining pH is essential for good staining in malaria diagnosis, particularly when the diluent is distilled water or tap water A simple and convenient apparatus is the B D H Comparator Set with bromo thymol blue as the indicator * Bromo thymol blue has a sharp colour change from yellow to blue over the pH range 6 0 to 7 6 with a greenish blue at 7 0 to 7 2 which can be matched accurately to 0 1 The method of estimation is as follows

(*a*) Add 1 0 ml of a one per cent solution of bromo thymol blue to 9 0 ml of the distilled water or solution for which the pH is required

(*b*) Match against the colour standards of the set

(*c*) Adjust if necessary to pH 7 0–7 2 for Romanowsky staining by adding a few drops of a saturated solution of lithium carbonate

Buffer solutions—Buffer solutions contain acidic and basic salts in proportions which can be adjusted to a definite and stable pH Accidental additions of small amounts of acid or alkali do not alter this pH the solutions are hence said to have a buffer effect against pH change The use of buffer solutions as a diluent for Giemsa stain first recommended by Coenaes (1927) has since proved to be a useful advance in technique The usual buffer salts are the alkaline and acid phosphates potassium dihydrogen phosphate and disodium hydrogen phosphate The phosphate buffer solution used in this Institute has the formula

Potassium dihydrogen phosphate KH_2PO_4	0 7 g
Disodium hydrogen phosphate Na_2HPO_4 (anhydrous)	1 0 g
Distilled water	1 000 ml

* List d by B ti h Drug Hou Ltd th indicat b mo-thym l bl hould be pecified when ordering The colour tand d t th B D H Comp t S end to f de in th tropics some workers f this eason p ef t its permanent gla c lour tand d th LOVIBOND Comp t also li ed by British Drug Houses has permanent glass tandards

COENAES, J (1927) Ov G ms kleuring v n bloedpraepar te N d *Tijds hr Gene k* Nov 5th 71 year p 934 (Ab tract *T p Di B ll* 19 8 25 486)

The pH of the solution normally 7 2 should be tested once on preparation and adjusted if necessary by further addition of one or other of the salts Disodium hydrogen phosphate tends to change when exposed to the air and an initial checking of the pH is a necessary safeguard Once adjusted the pH of the solutions remains the same for many months

GIEMSA STAIN

Originally manufactured in Germany from azures prepared by a secret process Giemsa stain may be prepared *from the original formula recommended by Giemsa* which is

Azure II eosin	3 0 g
Azure II	0 8 g
Glycerine	250 g
Methyl alcohol	250 g

Azure II eosin and Azure II are available already mixed in the correct proportions Solutions are conveniently prepared from this complete Giemsa stain *

The stability of Giemsa stain related apparently to the presence of the glycerine is an important advantage particularly in the tropics where Leishman stain may deteriorate within a few weeks No serious deterioration has been observed in Giemsa stain kept on a laboratory shelf in Kuala Lumpur for a couple of years

Preparation—The method of preparing Giemsa solution outlined below is used in the Malaria Division of this Institute The reagents required are—Giemsa stain 3 8 gm methyl alcohol 250 ml and glycerine 250 ml †

(*a*) Place 3 8 g of Giemsa stain in a mortar and grind thoroughly

(*b*) Add the glycerine to the mortar a little at a time grinding and mixing with each addition

(*c*) Add about half of the methyl alcohol continuing to grind and mix

(*d*) Pour the contents of the mortar into a glass stoppered Pyrex bottle

(*e*) Pour the remainder of the methyl alcohol into the mortar mix and grind up the residue of the stain and transfer the whole contents of the mortar to the bottle

(*f*) Label the bottle with the name of the stain the batch number and the date of preparation then incubate for 24 hours at 37 C giving the bottle an occasional shake

(*g*) Filter for use into dropping bottles

Stain would normally be prepared in this quantity only in busy laboratories Where the demand is small the purchase of ready made stain solution is convenient It has been our experience however that stain solutions made fresh in the laboratory are more reliable‡

Staining method for thin films—All methods of staining thin blood films with Giemsa stain have the common factor that the films must be fixed before the diluted stain is added They differ mainly in the duration of staining and in the concentration of stain The period of staining depends on the nature of the examination and on the time one can afford to wait Light staining is probably best for haematological studies but fairly intense staining with full

* Th t m ppl d mixtur f m thyl blue d ur ar omewh onf g Azur I (Giem) trad nam f ecr p p n whi h pp ar b ar bl mixtur f azur A d B Azur II (G ms) an al mixtur of q l par f Az I nd m thyl blue Azur II si mix f th eos na e f A ur II F full coun f hes ts h read *ef d Con H J B l gi l S ns 5th Ed B ot ch Public ns Gen New Y k 946*

† Th ar th ual p opor f th methyl lcoh l d glycer m w k rs p f proporti f 75 5 th p opor on G m rigi al f rmul was 50-5 by w gh 60-4 by lum

‡ Th ai will b inf if th m thyl lcoh l d glycerin ar t f h gh quality Th bes guara ee gainst tr bl f m thi our th f g f certifi d purity Bri h h micals f analytical gen ta dard w form ly nd ca d by th letter AR b th rm wa mewha indiscrimin nd om firm h d p d th rm ANALAR indica th tand d f p rity M thyl alcoh l (ANALAR co tai m than .05 p ce f on and 00 pe ent, f l til ma Th us f g f ANALAR quality trongly comme d d

differentiation is desirable for malaria diagnosis With diluted stain containing three drops of Giemsa solution to every millilitre of diluent, the parasites and their host cells are well stained in half an hour The method used in the Malaria Research Division of this Institute is outlined below

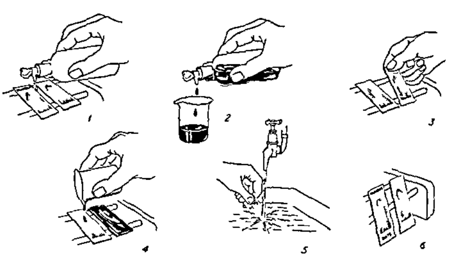


FIG 30—A GIEMSA STAINING METHOD FOR THIN BLOOD FILMS

(*a*) First prepare the diluted stain by dropping Giemsa solution into phosphate buffer solution in a clean vessel—3 drops of stain for every millilitre of buffer solution. One slide requires about 3 ml of the diluted stain (fig 30-2)

(*b*) Then fix the film with a few drops of methyl alcohol from a drop bottle (fig 30-1)

(*c*) After a few seconds—the time is not critical—pour off the alcohol and before the film dries pour on the diluted stain (fig 30-3 and 4)

(*d*) Stain for 30 minutes

(*e*) Differentiate by holding the slide film uppermost, under a brisk stream of water and leave it under this stream for fifteen seconds Never pour off the stain flush it off (fig 30 5)

(*f*) Place the slide on end to drain and dry (fig 30-6)

This method gives a fairly intense staining with a good definition of Maurer s Schuffner s, and often of Ziemann s stippling It is suitable not only for thin blood films but also for thin smears of brain, spleen, and bone marrow

Duration of staining—The depth of Romanowsky staining in blood films is determined by a number of variable factors among which the concentration of the stain and the duration of staining are important Within limits the effects of these two variables are inversely related the stronger the stain the shorter the duration of staining to achieve the same depth of colour In practice it is convenient to standardise the concentration of stain, our practice in Kuala *Lumpur is to stain thin blood films with a diluted stain containing three drops of* Giemsa solution (or stabilised Leishman solution) to one millilitre of phosphate buffer solution When films are stained for half an hour with diluted stain at this concentration the parasites are deeply stained they are well contrasted against straw pink erythrocytes and the stippling of infected cells is well defined The time may be shortened at need, indeed the parasites are

visible after five minutes staining but they are pale and the host cells are unstippled Species diagnosis in these circumstances may be difficult and very young trophozoites may be missed altogether

Duration of fixation—For malaria diagnosis the duration of fixation with methyl alcohol is not important it may be as short as a few seconds or as long as many minutes We are accustomed in Kuala Lumpur to leave the methyl alcohol on the films while the diluted stain is being prepared a proceeding which usually takes about a minute but may be longer when a large batch of films is being stained Rapid fixation has the advantage that it gives a better definition of polychromatic staining in immature erythrocytes but the minor drawback that it contributes to the production of the distorted erythrocytes known as demi lune cell (plate XXXIV) It is well to remember that methyl alcohol which has been exposed to the air may lose some of its activity as a fixative and that stock bottles containing methyl alcohol should be kept well corked

Colour contrast—Once a film is deeply enough stained the final colour depends in the duration of the wash If the red cells are too blue the wash has probably been too short if the red cells leucocytes and parasites are too pale the wash may well have been overdone The aim is to take as much colour as possible out of the red cells before the parasites and leucocytes have begun to lose their colour The pH of the wash water within the limits of domestic supplies is not critical Washing with distilled water is unnecessary

Criteria of good staining—Good staining of thin blood films for malaria diagnosis whether by Giemsa s or any other method should meet the following demands

(*a*) Freedom from debris pale straw pink red blood cells

(*b*) Polymorphs with deep purple nuclei and well defined granules

(*c*) Chromatin of malaria parasites a deep purplish red and cytoplasm a clear pale purplish blue

(*d*) Schuffner s dots in all enlarged cells containing *Plasmodium vivax* and Maurer s spots in cells containing the large rings of *P falciparum*

The colour of the nuclei of the leucocytes is a useful quick criterion the smallest falciparum rings will probably be missed if the leucocyte nuclei are not stained a deep purple

Giemsa staining of tissue dabs and smears—Preparations for malaria diagnosis whether of blood or of other tissues are best stained by a thin film technique with preliminary methyl alcohol fixation *if they are thin enough to be treated as films* The preservation of the red blood cells implicit in the thin film technique is an advantage not to be lightly discarded Smears on the other hand, should be stained without fixation by a thick film method the red blood cells are laked but the greater transparency of the smears compensates for this loss The method outlined below is used in this Institute it is the same in all essentials as the Giemsa staining method for thick blood films

(*a*) When the smears are thoroughly dry place them in a staining trough (fig 31 1)

(*b*) Add 33 drops of Giemsa stain to 100 ml of phosphate buffer solution (fig 31 2)

(*c*) Pour the diluted stain into the trough and leave for one hour (fig 31 3)

(*d*) Pour clean water into the trough to float off the scum (fig 31 4)

(*e*) Remove the slides one by one and wash by waving gently for 2 or 3 seconds in clean tap water (fig 31 5)

(*f*) Stand the films on end to drain and dry (fig 31 6)

The mutual adjustment of stain concentration and time of staining varies in different laboratories Some workers favour deeper staining than is recommended here—one drop of stain to one millilitre of phosphate buffer solution for the same or a lesser period This is a

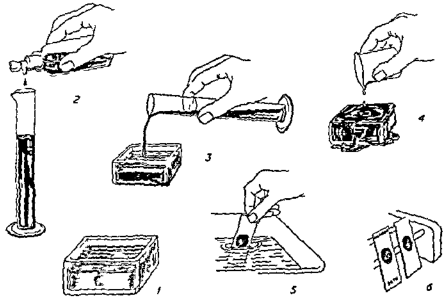


FIG 31—A GIEMSA STAINING METHOD FOR SMEARS OF BLOOD SPLEEN BONE MARROW, AND PLACENTA

matter of personal preference Deep staining darkens the parasites but also darkens the background

Shute (1955) has drawn attention to the more complete lysis of the red cells and hence the cleaner thick film background which is obtainable when a little common salt is added to the stain The salt he suggests, ensures a more complete solution of the haemoglobin Our early trials of this method have been very favourable Not only is the background of the stained thick film cleaner than that obtained with ordinary Giemsa staining, but Schuffner's dots are better defined For this improved Giemsa staining the procedure outlined above may be modified as follows

(a) Prepare the phosphate buffer solution (p 96) by dissolving the phosphate buffer salts in normal saline (0 85 per cent sodium chloride) instead of distilled water

(b) Use a more concentrated stain—100 drops of Giemsa stain to 100 ml of buffer solution instead of 33 drops

LEISHMAN STAIN

Used extensively in the British colonies over the past fifty years Leishman stain perhaps more than any other has brought to students of malaria their first acquaintance with the malaria parasites Wright stain popular in America is essentially the same and in this account the two names will be regarded as synonymous Leishman stain like Giemsa stain is a complex mixture of the eosinates of methylene blue and azure, it differs in that the solvent for the stain is methyl

SHUTE P G (1955) An improved technique for staining blood films with Giemsa stain *Nature Lond* 175 727
— AND MARYON M (1955) *Trans R Soc trop Med Hyg* 49 306

alcohol without glycerine Leishman stain being a methanolic solution is thus a fixative also and in the conventional method of staining the films are fixed with the undiluted stain

Preparation—Leishman stain is supplied by manufacturers either in solution or in dry form for the preparation of the stain solution in the laboratory Our experience has been that the stain is better prepared fresh in the laboratory and we would advise against the purchase of prepared solutions The reagents needed are Leishman powder 0 15 g and methyl alcohol 100 ml A simple and reliable method of preparation is recommended by James (1929)

(*a*) Add 0 15 g Leishman powder to a glass stoppered bottle which has been previously rinsed with methyl alcohol
(*b*) Add 100 ml methyl alcohol (ANALAR) to the bottle
(*c*) Shake two or three times over 24 hours
(*d*) Keep in a cool place
(*e*) Use without filtration

Staining method for thin films—The method outlined below is the conventional method of staining thin blood films with Leishman stain

(*a*) Add 4 drops of stain to the film
(*b*) After 15 seconds add 12 drops of buffer phosphate solution tilt and rock the slide gently to mix stain and diluent evenly *
(*c*) Stain for 30 minutes
(*d*) Flush off the stain for a few seconds under the tap
(*e*) Place the lide on end to drain and dry

The time of staining may be shortened to as little as ten minutes but a loss in the quality of staining is then to be expected The stippling of infected cells is slower to stain than the parasites and a shortened staining time may fail to bring out the characteristic appearances of the infected cells

Staining method for gametocytes—The deep staining which best brings out the stippling of malaria infected cells tends to mask the differences of sex in falciparum gametocytes For an easier distinction between male and female Shute and Maryon (1951) recommend that the Leishman stained films be left to soak for a few minutes in a jar of distilled water This procedure washes some of the colour from the gametocytes without decolourising the chromatin the cytoplasm or the host cell and it emphasises the differences of sex The chromatin of the female gametocyte shows up as a compact solid block and the pigment which usually surrounds the chromatin can be seen more easily The pigment granules around the chromatin of the male are similarly well defined

A STABLE LEISHMAN STAIN

While Leishman staining at its best is unsurpassed for malaria diagnosis from thin films and the colours of the stained parasites are perhaps richer than those obtained from Giemsa staining the Leishman method has several drawbacks in tropical countries

(*a*) The stain is unstable a useful life of a few weeks is all that can be expected in tropical countries
(*b*) The technique cannot be precisely standardised

I th Sh chnique th dil d ll d wa dj ted t pH 7 by th ddi o f f w d ops f tu d l tio of lithi m carb na

JAME S P (9 9) A c th Sh hniq f tai ing m l ri p es w h L hma ai d h ppl g n inf ted d bl od corpuscl whi h t al *T R Soc p M d Hyg* *23* 69

SHUT P G AND M N M (95) A tudy f g m tocyt W Af ican ai f *Plasmod um f l p rum T ns R S p M d H g* *44* 4

(c) Debris in the stain tends to be fixed in the plasma proteins when the films are fixed in the undiluted stain and cannot be washed away

These objections may be met by stabilising the stain with glycerine and, since the stain is no longer a fixative when glycerine is added to it by fixing the films with methyl alcohol Prepared with the addition of glycerine Leishman stain is no less stable than Giemsa stain a batch of stable Leishman stain prepared in 1949 and kept on a laboratory shelf in Kuala Lumpur was still a good stain in 1955 Most of the colour drawings which appear as plates XXXVI to LV were made from films stained by this modified Leishman method

The reagents for stabilised Leishman stain are Leishman powder 3 8 g methyl alcohol (ANALAR), 250 ml and glycerine (ANALAR) 250 ml The method of preparation is that outlined on p 97 for Giemsa stain using Leishman powder instead of Giemsa powder The method of staining thin blood films is the same as the Giemsa thin film method described on p 98, using stabilised Leishman solution instead of Giemsa solution

THE PREPARATION OF FILMS AND SMEARS

Films or smears for malaria diagnosis are nearly always made from the blood exceptionally they may be prepared from the bone marrow spleen or skin, after death they may be taken also from the brain

Blood films—For the preparation of thin films, the blood is usually taken from the pulp of a finger, the nail bed, or the lobe of an ear The finger pulp is convenient though some workers favour the nail bed just below the lower visible edge of the finger nail or the tip of an ear lobe The tiny fingers of infants are less convenient as a source of blood than the pulp of a

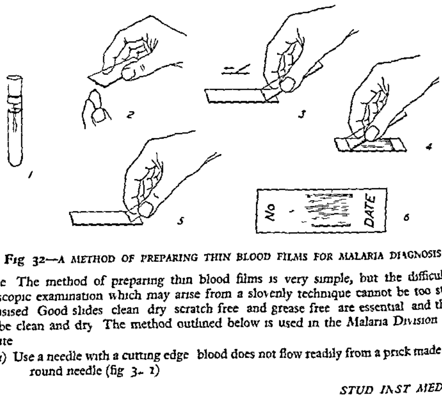


Fig 32—A METHOD OF PREPARING THIN BLOOD FILMS FOR MALARIA DIAGNOSIS

big toe The method of preparing thin blood films is very simple, but the difficulties in microscopic examination which may arise from a slovenly technique cannot be too strongly emphasised Good slides clean dry scratch free and grease free are essential and the skin must be clean and dry The method outlined below is used in the Malaria Division of this Institute

(a) Use a needle with a cutting edge blood does not flow readily from a prick made with a round needle (fig 32 1)

(*b*) Clean the pulp of a finger with alcohol using a piece of gauze or a pledget of cotton wool wait till the finger is dry and then prick with a needle

(*c*) Squeeze out a tiny globule of blood—the blood remains as a globule if the finger is dry if the blood spreads over the finger wipe it away and squeeze again—and touch the globule with the end edge of a slide or cover slip spreader (fig 32 2) Slides with ground edges make good spreaders even better are rectangular cover slips (fig 32 5)

(*d*) Apply the spreader to the slide at an angle of about 45 , wait till the blood extends along the spreader edge for about two thirds of the slide width then keeping even contact push the spreader forward along the slide (fig 32 3 and 4)

(*e*) Dry the film quickly by waving the slide in the air

(*f*) Label the slide with the date and the name or number of the patient

Good blood films should be evenly spread and free from streaks or holes The end of the spread should taper to a point or else be convex outwards and it is well to remember that malaria parasites and their host cells are often best stained at this convex end of the spread Within limits the thickness of the films may be controlled by the speed of spread a film spread quickly is likely to be rather thick a slow spread on the other hand tends to yield a very thin film Drying the films quickly by waving the slides vigorously in the air tends to improve the quality films which dry slowly as they are apt to do in damp weather do not always stain well the red blood cells moreover may be semi laked by the slight moisture on the slide Lysis may be prevented even in the dampest days by passing the slides over the flame of a spirit lamp to drive off the moisture before the films are taken Some of the common faults in the preparation of films are illustrated in figure 33 The globule of blood should be very small if the amount of

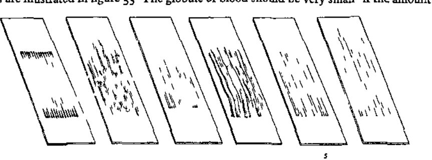


Fig 33—SOME COMMON FAULTS IN THE PREPARATION OF BLOOD FILMS

blood is excessive the end of the spread will be lost (fig 33 1) Blood does not spread evenly on greasy slides (fig 33 2) and a notched spreader or an uneven contact between spreader and slide leads to a streaky film (fig 33 3) There should be no undue delay in spreading the films or they may be messy from early fibrin formation (fig 33 4) Films from anaemic blood tend to be very thin and to extend far up the slide (fig 33 6) a defect which can be corrected to some extent by a very quick spread One should aim to make the films a little narrower than the slide and from half to two-thirds as long with the end of the spread well short of the end of the slide (fig 33 5)

Fresh blood—The examination of fresh unstained fluid blood for malaria diagnosis is necessary only under exceptional circumstances when for example no stains are at hand but a study of the living organism is nevertheless a useful exercise for students of malaria

The simplest method of making fresh fluid blood films is to place a minute drop of blood on the centre of a coverslip and then to let the inverted slip fall gently on a slide The drop must be so small that the blood does not *quite extend to the edge of the slip* For an even spread both slide and coverslip must be clean and grease free The best zone for examination is the middle zone (fig 34C)

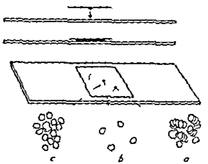


FIG 34—THE DISTRIBUTION OF RED BLOOD CORPUSCLES IN A FRESH FLUID FILM UNDER A COVERSLIP (after Daniels and Newham 1923)

Another useful method is that recommended by Braddon A square coverslip, free from grease is placed on a grease free slide with one edge corresponding with an edge of the slide With the *coverslip pressed against the slide the edges are sealed with vaseline leaving* unsealed the outside edge and a small space at the opposite edge (fig 35) For the preparation of the film the unsealed edge is applied to a drop of blood the blood drawn by capillary attraction, spreads as a thin film between coverslip and slide

Exflagellating Gametocytes—Exflagellation in the male gametocytes may be studied either in fresh blood or in stained smears *Fresh preparations are essential for studies of the* activity of the gametocytes up to the formation and release of the gametes stained preparations have the advantage of permanence

Fresh preparations—A simple method of studying exflagellation was shown to one of us (P G S) by J W W Stephens many years ago Prick the finger and let a tiny bead of blood *touch the centre of a clean grease free coverslip Breath on the* coverslip quite *heavily* and then invert it and let it fall on a clean grease free slide The moisture helps the blood to spread out one cell deep Have at hand a spirit lamp, a little vaseline in a shallow vessel and a match stick or camel hair brush and as soon as *the coverslip makes contact with the slide* heat the vaseline and run a seal round the coverslip

Stained preparations—Stained preparations of exflagellating gametocytes are best made from thin films which have been kept for a few minutes in a moist chamber prepared from a petri dish lined with moist filter paper Fit two layers of filter paper accurately cut to size in both halves of each of four *petri dishes 3½ inches in diameter Lay a triangular* piece of glass tubing in the bottom of each dish Half an hour before the preparations are to be made moisten the filter paper in each dish with hot water Prick the finger, make a thin film, breathe on it *lightly, place the slide on the glass tubing in* one of the dishes and *close the dish* Repeat the process for the other three dishes Take out a slide after ten minutes and the remainder at 3 5 minute intervals Dry the films by waving the slides in the air or by passing them film uppermost, across the *flame* of a spirit lamp Fix with methyl alcohol and stain with *Leishman*

DANIELS C. W AND NEWHAM H B *Labor t ry tudies T op cal M di in* 5th d. John B le S ns and Danı l on Ltd London 1923

or Giemsa stain in the usual way In temperate or cold climates the petri dishes should be kept in an incubator and the films should be removed from the moist chamber at five minutes intervals This is essentially the method of Shute (James 1934)

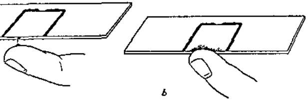


Fig 35—BRADDON S METHOD OF PREPARING FRESH FILMS OF FLUID BLOOD (after Daniels and Newham 1923)

Blood clot—Thin films suitable for malaria diagnosis cannot be made direct from blood clot smears are rather messy and the texture of clot is unsuitable for the squash preparations which yield such excellent films of brain tissue Perhaps the most useful preparations are

(*a*) dab smears made by a single dab of a small piece of clot on a glass slide and stained without fixation by a smear technique (p 99) or

(*b*) films made from the centrifuged deposit by the method of Denes (1931) The serum having been removed with a pipette the clot is shaken up in the test tube and then discarded the dregs are spun in a centrifuge films are made from the deposit dried and stained by a thin film technique

Brain—Brain tissue may be removed either by needle autopsy through the orbit or from the exposed brain and it may be examined for malaria diagnosis (*a*) in fresh squash prepara tions (*b*) in stained thin smears or (*c*) in stained sections Histological examination is omitted from this account

Needle autopsy—When a full autopsy examination is not possible enough brain tissue for microscopic examination may be got by the method of needle autopsy suggested by Daniels and Newham (1923) A stout exploring needle at least 3 long is inserted through the conjunctiva under the upper eyelid into the orbit and pushed upwards through the orbital plate into the brain Suction with a syringe will draw a small piece of brain into the needle which is then withdrawn In some cases suction may be unnecessary as brain tissue is forced into the needle in its passage The brain tissue is expelled from the needle on to a slide and fresh or stained preparations are made as described below

Fresh squash preparations—Place a small piece of brain about the size of a small pin s head on a slide cover with a slip and gently press down until the brain tissue spreads thin to the edge of the slip Examine fresh first with a 1/6th dry and then with a 1/12th oil immersion objective Low power examination will often show the minute masses of malaria pigment delineating the lines of the capillaries the high power may reveal the capillaries packed with parasites in active movement Brain examined in the tropics many hours after death may still show parasites with a lively movement in the pigment and apparently still alive

JAMES S P (1934) Th Sh m th d f mak g p p ti ns f exfl g ll ting g m ocyt and oök f malar al p as es *T ns R S p M d Hyg* 28 4

DENE G (193) R het h d paras m lar que d l ang *B ll S n I I S I ern d M cr biol gi* Milan 3 66 (Ab tr *T p D B ll* 93 29 355)

DANIELS N N WHAM 9 3 (f p 4)

Stained 'squash' smears—Excellent smears may be prepared by a modification of the method suggested by Raja (1922) A piece of brain about the size of a small pin s head is placed in the centre of a glass slide, and covered with a second slide applied at an obtuse angle The two slides are gently squeezed together until the brain tissue spreads to the edge They are then abruptly pulled apart in the plane of their surfaces Two thin smears are formed in this way, one on each slide They are dried, fixed with methyl alcohol, and stained by a thin film technique The distribution of parasites in the brain is sometimes uneven, tissue should hence be examined from several parts of the cortex basal nuclei, and cerebellum

The capillaries in these 'squash' smears are well preserved and can often be followed for a considerable distance, while the preservation of the parasites and of some of the histological detail is usually very good We may perhaps note that brain smears should not be made by taking a piece of brain and smearing it along a slide, this method, though appropriate for spleen and placenta, yields very poor preparations of brain tissue

Spleen—Material from the spleen may be obtained by needle biopsy, or after death by direct examination of the spleen pulp Spleen biopsy is seldom practised to-day for malaria diagnosis, the procedure is not free from danger and we are inclined to accept the views of those who believe that the information to be gained does not justify the risks small though they may be The technique and the precautions to be taken are described in most text books of tropical medicine, particularly in relation to the microscopic diagnosis of kala azar

After death, spleen pulp may be withdrawn with a syringe and a large dry exploring needle A small portion from the tip of the needle may be spread on a slide as a thin film, using a second slide as a spreader the rest may be used to prepare smears The films should be fixed with methyl alcohol and stained by a thin film technique, the smears should be stained without fixation

When a full post mortem examination is possible films and smears should be made from the freshly cut surface of the spleen Films may be prepared by taking a surface scraping of pulp and blood with the end edge of a slide, and quickly spreading the material before it dries on a second slide The films should be made as thin as possible, the dilution of a little spleen pulp in a pipette with an equal volume of citrate saline and the preparation of films from th suspension may be a help in this direction Smears are conveniently made by a 'dab' technique, a small piece of freshly cut spleen is taken up with forceps and dabbed once on the surface of a slide As with other tissue preparations, the films should be fixed with methyl alcohol before staining, and the smears should be stained without fixation.

Bone marrow—Aspiration biopsy of the bone marrow has been recommended by some workers as a useful ancillary method of malaria diagnosis (*see* p 82) We have had no experience of this method For an account of the technique of marrow biopsy the reader may refer to any modern textbook of haematology

Placenta—Films and smears from the placenta are best made from a freshly-cut surface For the preparation of films, blood may be taken by a light scraping of the surface with the end edge of a slide—or better still with the end edge of a rectangular coverslip—and quickly spread on a second slide A better method perhaps is to prepare the films from placental blood which has been mixed with an equal volume of citrate saline A cut is made into the maternal surface of the placenta and into the notch so formed a few drops of citrate saline are introduced with a pipette, blood and saline are mixed in the notch by drawing the mixture up and down in the pipette Thin films are prepared from the mixture fixed with methyl alcohol and stained with

RAJA R N (1922) Post mortem examination in cerebral malaria A new and simple method of demonstrating parasites in the capillaries of the brain *Ind med Ga* 57 298

Leishman or Giemsa stain A common error is making thin films from the placenta as indeed from other tissues is to take too much blood The amount needed is exceedingly small—a drop the size of a small pin s head is usually excessive

Smears are conveniently made by a dab technique A small piece of placenta freshly cut from the maternal surface and held with forceps is dabbed once on the slide leaving behind a smear with the cellular elements relatively undistorted Smears should be stained unfixed by an appropriate thick film method (*see* p 99)

Skin—Skin scarification smears have been used in the Belgian Congo for routine malaria diagnosis The method is described by Van den Berghe and Chardome (1951) The scarification is practised on the middle of the scapula region where the skin is rather poorly irrigated and presents thick layers of subcutaneous tissue In order to be limited if possible to the dermal layers it must be superficial barely cutting the dermal capillaries and lymphatics A deep cutting would produce more bleeding and be less satisfactory for examination of the dermis If the scarification has been done properly little or no blood should appear before the squeezing of the scarified site In making the preparation the scarified skin must be thoroughly squeezed between the thumbs and forefingers Serous fluid obtained in this manner is largely made up of dermal juice with the blood extravasating only in small amount out of the dermal capillaries The fluid is placed in the centre of a microscopic slide and spread either on a thin film of rather small size or in the same manner as a thick drop of blood The film is then stained as a thick blood preparation preferably by the Giemsa technic

PRESERVATION OF MATERIAL

All tissues containing malaria parasites whether fresh or stained tend to deteriorate with storage particularly in the tropics and sooner or later become useless for malaria diagnosis unless precautions are taken for their preservation The problem of preservation arises with fresh tissues unstained films or smears stained films or smears and viable parasites

Fresh tissues—Degenerative changes in blood brain spleen bone marrow and placenta the tissues from which an examination for malaria is most helpful appear soon after death or removal from the body In the cadaver state Marchiafava and Bignami referring to the ring forms of *P falciparum* we do not see annular bodies but only as a rule immobile discoid or spherical micrococciform bodies And even these bodies may become unrecognisable as malaria parasites after about 24 hours

Refrigeration is perhaps the most useful method of conservation The parasites and the cellular elements of the tissues may still be identified after one week—and probably for much longer though we have no data on this point Brain from two cases of cerebral malaria preserved by Mayer and Pifano (1949) in an ice box for nine years still revealed the parasites and cellular elements in a good state of preservation (The prolonged preservation which is possible in paraffin blocks prepared for histological examination is outside the scope of this account)

Unstained films and smears—Thin blood films whether fixed or unfixed cannot normally be kept unstained at room temperature in the tropics for more than a few days without serious deterioration They stain best within the first forty-eight hours when they are stained after several days the erythrocytes are slate grey and with prolonged delay they are blue and the colour contrasts within the parasites are degraded At first slight the loss in staining quality

VAN EN BER HE L AND CHARDOME, M (95) An ier d m cur diagnos f malar d filari th ough th use f th kin carifica m ar *Am J p M d 31* 4

MAYER M AND PI AN C F (949) C ns rv ció por nos en l de er b os *Pl sm d um fal p rum* (Ab t ct *T p D B ll* 95 47 59)

becomes marked after four or five days Smears of blood and of other tissues show a similar deterioration when the staining is delayed much beyond twenty four hours the red blood cells fail to lyse properly and the stained smears have a bluish background against which the parasites are poorly contrasted Shute (1946) has drawn attention to the fact that films kept at 50 F stain better than those kept at 80 to 90 F, and refrigeration alone seems to be a partial answer to the problem A better method of preservation is that of Jaswant Singh *et al* (1949) who combined refrigeration with dessication We have kept unstained thin films both fixed and unfixed in a dessicator placed within a domestic refrigerator, for two years with no serious loss in their staining quality Blood smears have been similarly stored with little loss in their staining properties For tissue smears we have no data, but there seems to be no obvious reason why the method should not serve equally well for tissues other than blood

The dried unstained films are conveniently stored wrapped in long strips of paper three inches wide and so folded, backwards and forwards, that the slides are kept apart by a single thickness of the paper the package of slides being secured by a rubber band When the films are needed, the dessicator must be removed from the refrigerator an hour or two beforehand in order that the contents may be at room temperature when the dessicator is opened, failure to observe this precaution may lead to a deposit of moisture on the cold slides with haemolysis of the films

Stained films and smears—The preservation of colour in Romanowsky stained films is a curiously variable phenomenon Shute has kept stained films at laboratory temperature in London with no loss of colour in thirty years Films kept in tropical climates on the other hand may fade in a few months but even here there are curious variations for some stained films retain their colour for years while others stained in the same way from the same batch of stain begin to fade after a few weeks Fading is hastened by mounting media by the presence of immersion oil and by exposure to light Not only do films tend to lose their colour but if they are not kept dry they are liable to contamination by moulds

Here again refrigeration with dessication is probably the best safeguard Trials of this method began in Kuala Lumpur two years ago, no fading or other deterioration is yet apparent

Other methods for preserving the colour of Romanowsky stained films and smears have been recommended by Pawan (1935) and by Clark and Tomlinson (1949) By the method of Pawan the slides are dipped momentarily into a saturated solution of paraffin wax in xylol the xylol evaporates and the slides are left covered with a thin layer of paraffin When the films are wanted, the paraffin is dissolved away by immersing the slides in xylol Treated in this way films have been kept in Trinidad for five years with their staining unimpaired

Clark and Tomlinson have reduced fading by immersing the stained slides successively in xylol and in toluol for a few minutes each and then mounting the films under a coverslip in neutral synthetic resin Films treated in this manner have shown no evidence of fading after being kept on the top of an incubator at 35 C for six months

A method of protecting stained thin films from damage by coating them with cellulose lacquer is recommended by Tate (1933) A suitable commercial preparation Durofix is sold in collapsible tubes A large drop of Durofix is extruded on to one end of the slide and wiped over the film with a sweep of a clean finger

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SHUTE P G (1946) Rev w f an rti le by Ra liff A W (1946) *T p Dt Bull* 43 967
JASWANT SINGH RAY A P AND N C. P (1949) A p liminary on th pres rv ti f unsta d blood smears *I d J M l* 3 327
PAWAN J L (1935) Th p rv ti n in th t p f blood mears tain d by th R ma w ky tain. *A tr p Med. P sit* 29 3 7
CLARK AND TOMLINSON 1949 (f p 84)
TATE T (1933) A me hod f l b lli g d p tecti g blood films *T ns R S t p M d H g* 31 118

Viable parasites—When infected blood is taken from the body and kept at room temperature the parasites soon die but at low temperatures they may survive for many days even for months The preservation of viable parasites has mainly an experimental application A simple method of keeping the parasites alive for a few days is to store the infected heparinised blood in a test tube with suitable refrigeration the addition of a little glucose to the blood may contribute to a longer survival The tube is placed in a vacuum flask surrounded by chipped ice which should be changed every 24 hours By this method Dr T Wilson and Dr J F B Edeson have sent blood infected with *P falciparum* *P malariae* or *P knowlesi* by air from Kuala Lumpur to Delhi Nairobi or London where the parasites have been established in fresh hosts up to five days later

By rapid freezing and the maintenance of a temperature between—70 C and—80 C using dry ice in a Horsfall apparatus Coggeshall (1939) has been able to preserve *P knowlesi* for 70 days and Boyd using a similar technique has maintained the viability of *P falciparum* and *P vivax* for 156 days

CONCENTRATION METHODS FOR MALARIA PARASITES IN BLOOD

Some of the methods used to concentrate malaria parasites in blood for diagnostic or other purposes were briefly reviewed in an earlier chapter They fall broadly into two groups—those which depend on a differential layering of infected cells in blood which is allowed to sediment or else spun in a centrifuge and those for which the blood is laked and the parasites are concentrated in the centrifuged deposit The first method fails to concentrate the smaller parasites particularly the young rings of *P falciparum* the latter is said to concentrate falciparum ring forms but the red cells are destroyed and in our hands the results have been erratic Two representative methods are outlined below references to accounts of other methods are given in chapter VII (p 89)

Bass and Johns method—The earliest method for concentrating malaria parasites in blood and still one of the best methods is that of Bass and Johns (1915) The method is very effective for crescents and other large parasites but fails with young falciparum rings

Apparatus

1 10 ml glass syringe
2 1 ml pipette graduated in hundredths
3 Centrifuge tubes about 1 5 cm inside diameter and 12 cm long
4 Centrifuge tubes 0 5 cm inside diameter and 12 cm long—made from suitable glass tubing
5 Large capillary pipettes—inside diameter about 0 1 cm
6 Small capillary pipettes—outside diameter less than the inside diameter of the large pipettes
7 Rubber teats glass cutting file
8 Centrifuge with a speed of 2 000 revolutions or more per minute

Technique

(*a*) Draw 10 ml of blood from a vein and expel it into a test tube containing 0 2 ml of citrate dextrose solution* revolve and shake the tube to mix

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C dextr l —sodi m ra 5 g d xt os 5 g in 00 ml di till d w te

COG AL L T (939) P f bl mal ria par es in th f ze ta *Proc S p B l M d 4* 499 (Ci d by Brump 949)

B YD M F (Ci d by Brump 949)

B M 949 (f p 8)

BASS AND J HN 9 5 (f p 89)

(b) Put equal quantities of the citrated blood into two large centrifuge tubes and spin at 2 500 r p m for 3 to 5 minutes (one minute for each cm of the column of blood)

(c) With a large pipette, skim off from both tubes the top layer of the sedimented cells—consisting of red cells, leucocytes and the larger parasites—take up an equal volume of plasma, expel into a small centrifuge tube and mix by drawing up and down in the pipette The column in the small tube should not exceed 5 cm

(d) Spin the small tube in a centrifuge as before

(e) From the top layer of the sedimented cells in the small tube, draw up a 5 cm column into a large capillary pipette mix by drawing up and down in the pipette on a slide draw up finally beyond the tip of the pipette, and seal the tip in a flame

(f) Cut off the capillary part of the pipette containing the blood and spin as before

(g) Cut the capillary tube at a point about 0 1 to 0 2 cm below the bottom of the leucocyte layer and with a small capillary pipette draw off the leucocytes and red blood cells with a little plasma Mix and prepare films or smears

Krauss and Fleming s method—A representative example of the methods of concentration which depend on a laking of the blood is that of Krauss and Fleming (1916)

Technique

(a) Prepare a citrate saline solution containing 2 0 gm sodium citrate (acid free) and 0 85 gm sodium chloride to 100 ml distilled water

(b) Draw 2 0 ml of blood into a syringe containing 0 5 ml of citrate saline solution and empty the citrated blood into a bottle containing 10 ml of the citrate saline solution

(c) Add 0 5 ml of a one in ten dilution of formalin (this preserves the parasites, small rings are said to be fixed in half an hour large parasites in 4 hours)

(d) Spin in a centrifuge at high speed for 10 15 minutes

(e) Pipette off the plasma with the uppermost layer of leucocytes discard and replace by distilled water shake thoroughly and spin again as before

(f) Gently pour off the contents of the tube leaving only the deposit

(g) Prepare films and smears from the deposit

CULTIVATION OF THE PARASITES IN VITRO

When malaria infected blood has left the body the parasites may continue to grow for a short time depending on the conditions, but they soon curl up and die Bass and Johns (191) were the first to show that the addition of a little dextrose to the blood promotes survival and that growth may continue through the complete schizogonic cycle or even a little beyond to the beginning of a second cycle Their method is simple and effective and though more elaborat methods designed for prolonged survival have been devised it remains perhaps the most useful method for the laboratory with limited time and resources

Equipment

A 50 per cent solution of chemically pure dextrose sterilised at 100 C on three successive days (fig 36 1)

A sterilised 10 or 20 cc glass syringe with a rather wide bore needle

KRAUS AND FLEMING 9 6 (r f p 80)
BASS AND JOHNS 9 (ef p 33)

Sterile defibrinating tubes plugged with cotton wool with a glass rod running through the wool to the bottom of the tube (fig 36 2*a*)

Sterile culture tubes not less than 1 .5 cm in diameter by 12 5 cm deep (fig 36 2*b*)

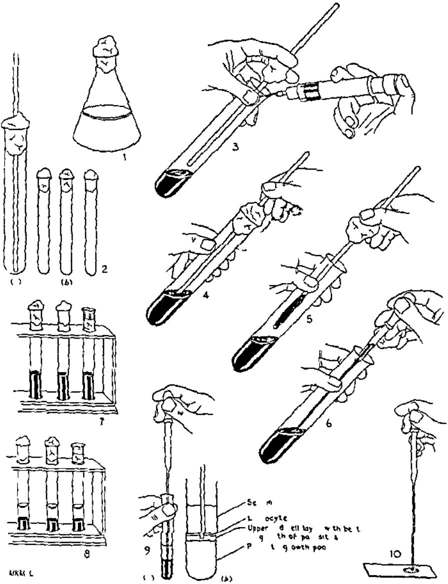


Fig 36—CULTIVATION OF MALARIA PARASITES BY BASS AND JOHNS METHOD (slightly modified)

Sterile rather coarse capillary pipettes

Incubator regulated to 40 C (but any temperature between 38 C and 40 C will give good growth)

Technique for cultivating a single generation

1 Add 0 1 cc of the dextrose solution to the defibrinating tube

2 Slowly draw 10 cc of venous blood and run it gently into the defibrinating tube, avoiding frothing and unnecessary exposure to the air (fig 36 3)

3 Defibrinate the blood by gently stirring with the glass rod (fig 36-4), after a few minutes withdraw the rod with the fibrin clot (fig 36 5), and close the tube with another cotton wool plug

4 With a pipette transfer the defibrinated dextrose blood to the culture tubes the column of blood in the culture tubes should be from 2 5 cm to 5 0 cm deep (fig 36-6 and 7) [We have sometimes found it advantageous to seal one of the tubes by pushing in the cotton wool plug for a short distance and then filling the top of the tube with melted paraffin (fig 36 7 and 8)]

5 Incubate at 40 C (or between 38 C and 40 C) the blood will separate during incubation into the layers indicated in fig 36 9b

6 With a pipette withdraw a small quantity of blood from the top layer of the sedimented cells for examination The culture tubes must always be kept upright, never tilted (fig 9)

The cultures are best examined after from 18 to 36 hours of incubation In removing the top layer from the surface of the sedimented cells in the culture tube it is convenient also to take up an equal volume of serum The cells and serum are gently mixed on a glass slide by drawing up and down in the pipette (fig 36 10), and the parasites are examined

(*a*) in fresh films under a coverslip prepared by placing a minute drop on a glass slide and covering with a slip,

(*b*) in films fixed with methyl alcohol and stained by a thin film method,

(*c*) in smears stained unfixed by a smear method

The phenomenon of clumping is best seen in the fresh preparations and smears in thin films the clumps tend to break at the end of the spread The phenomenon is particularly well shown in fresh preparations made by taking up a minute quantity of the top cell layer in the culture tube with three or four times this amount of serum the cells and serum are gently mixed on a glass slide by drawing up and down in the pipette, and a minute drop of the mixture is examined under a coverslip Clumps of infected cells containing parasites advanced in growth may sometimes fill the high power field of the microscope These clumps examined by dark ground illumination with the parasites in active movement and the pigment shimmering in light, give a lively impression of this remarkable phenomenon

PARASITE COUNTS

An estimation of the density of parasites in the peripheral circulation made before treatment has reduced the number, gives a useful indication of the severity of a malaria attack. A rough and ready method is to assess the average number of parasites in a microscopic field Assuming an average of 200 red cells in a thin film field, and a red cell count of 4 000 000 per c mm of blood the approximate density when there is one parasite per field would be 20 000 per c mm A quick clinical estimation would then be the average number of parasites per thin film field multiplied by 20 000

Another simple method like the first open to considerable error but clinically useful is to count the parasites against the leucocytes Taking an arbitrary density of 5 000 c mm for the leucocytes and counting the number of parasites and leucocytes in the same fields the approximate parasite density may be readily assessed The estimation would of course be more accurate were the leucocyte density known

A third method still more accurate and probably the best method when the parasite density is high, is that based on the total red cell count and the relative count of red cells and parasites This method is particularly recommended for its prognostic value in severe falciparum infections

Another figure of undoubted clinical value in falciparum malaria is the proportion of red blood cells infected With more than 10 per cent infected the prognosis is serious with 20 per cent it is grave with 30 per cent it is almost hopeless Recoveries have been recorded with 35 per cent infected but never so far as we know with a proportion higher than 35 per cent Only in falciparum malaria do the parasites ever attain these high densities

PLATES

PLATES I LIX

Source of material—With the exceptions acknowledged in the text the drawings are original They were made at the microscope from films prepared in the course of the day by day routine of the Malaria Research Division of this Institute The parasites studied again with a few exceptions were those found in blood films from Asian patients naturally infected with malaria in the Malay States of Selangor and Negri Sembilan

Staining method—The blood films were usually stained by the Leishman method outlined on page 102 the stain contained glycerine and was buffered to a pH of 7 2

Optical equipment—The optical equipment included

(a) a binocular research microscope
(b) a 1/16 fluorite oil immersion objective
(c) ×10 periplanetic eyepieces one containing a glass disc ruled in squares with the optical system used the squares of the grid gave a projection of approximately 10μ×10μ in the plane of the image
(d) a slide micrometer with 10μ divisions
(e) a concentrated filament light source with a light blue filter

Method of drawing—The microscopic field was plotted on a drawing sheet ruled in squares These squares tallied with the squares in one of the eyepieces the scale being prede termined by the slide micrometer Important structures were separately drawn at a magnification of ×2 900 The final colour drawings were sketched from the working drawings the colours being matched at the microscope

Magnification—The original drawings for most of the monochrome plates are reproduced at a magnification of ×2 250 and a direct comparison between the drawings on different plates will usually be possible There was occasional difficulty however with drawings from other sources as the scale was not always clear The magnification for the micros copic fields portrayed in the colour plates is ×1 300 with the exception of plate XLVI which is reproduced at ×1 100 Figure 37 compares the areas of (A) the usual microscopic field in routine examination for malaria (monocular microscope 1/12 immersion objective ×6 ocular) and (B) the field included in most of the colour plates

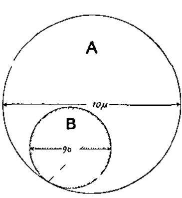


Fig 37—RELATIVE AREAS OF MICROSCOPIC FIELDS (*see* text)

Reproduction of colour—The final colours in a print produced by a simple three-colour process may deviate from the original microscopic image for various reasons The original drawing may fail to match the colours of the image losses or deviations may occur when three-colour blocks are prepared from the colour drawings and finally the balance of colour in the three stage printing of the plates may be at fault The colours in plates XXXVI to LIX are not true but we believe them to be fairly near to those of the original images The brilliant purples of Romanowsky stained material are not easily repro duced in colour prints partly for this reason partly from the losses which are inseparable from colour reproduction the plates tend to subdue the purples and blues and to over emphasise the reds

I THE ERYTHROCYTIC PARASITES GENERAL STRUCTURE

Two substances with a definite structure are always present in the malaria parasites, the body substance or cytoplasm and the nucleus Stained by the Romanowsky method the cytoplasm is pale blue in colour, the nucleus containing the red staining substance called chromatin, varies in colour from a pale pink to a deep red or purple depending on the amount and concentration of the chromatin the pH of the stain diluent, and other considerations Chromatin is the chromatic substance within the nucleus, but the term is also used loosely to mean the nucleus itself In the young erythrocytic parasites the cytoplasm and the nucleus are the only visible structures They are disposed in various shapes often appearing as a ring of cytoplasm with a nuclear bead somewhere on the periphery, and a clear space thought to be a nutritive vacuole in the centre (1)

When the parasite is about twelve hours old minute granules of pigment appear in the cytoplasm This pigment a by product of the metabolism of the organism, does not stain it has a natural yellowish colour, appearing in Romanowsky stained films to be pale yellow yellowish green or mahogany brown depending on the size of the pigment masses on the degree of masking by the blue stained cytoplasm and on various other factors The pigment granules increase in size and number as the parasite grows and are disposed in patterns which vary with the species and the phase of growth, in the later stages of schizogony they concentrate to a small area (3) or even coalesce into large masses

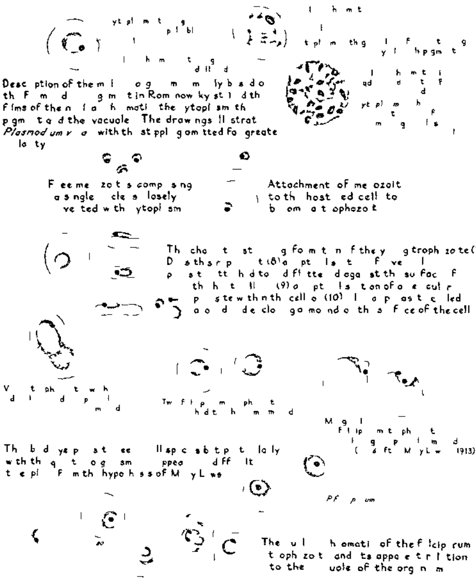
Some 36 60 hours from the first invasion of the erythrocytes—the time in the cycle varies with the species—the nucleus begins to divide, first into two, and finally into from half a dozen to twenty or more fragments, again depending partly on the species Figure 3 shows *P vivax* at an advanced stage of nuclear division

Cytoplasm nuclear chromatin and pigment are present in every advanced erythrocytic malaria parasite A good working rule in malaria diagnosis is to regard no red cell inclusion as a malaria parasite which does not clearly show at least two of these three structural elements

The young erythrocytic parasite often has the appearance of a ring enclosing a clear space with a nuclear bead somewhere on the periphery but the reality behind the appearance is not fully known The usual explanation is that the ring represents an optical section of a vesicular organism stretched and flattened against the upper or lower surface of the host cell Somewhere in the wall of the flattened vesicle lies the nucleus which for reasons unknown is usually sited at the side of the ring (7) But the nucleus may lie on the upper or lower surface and may then appear within the ring itself (17) On this hypothesis the clear area within the ring represents the red cell surface seen *through the thinned and stretched upper and lower walls of the vesicle* Another theory, suggested but not supported by Welch (1897) and developed particularly by Mary Lawson (1913) is that the ring represents a linear parasite coiled round a raised mound on the surface of the red cell The newly attached parasite she suggests throws out pseudopodia *which grip the* surface of the cell These pseudopodia into which flows most of the cytoplasm extend over the surface of the cell and meet to enclose a raised circular area from which the growing organism derives its food (10 16) On this theory the vacuole is nothing more than a circumscribed area of red cell surface These concepts are simply illustrated in plate I

WELCH 1897 (f p 46)
ROWLEY LAWSON MARY (1913) Th e tra llular lati f th malarial para t t th d blood corpuscl and ts method f uring ttachm t to th t rnal urf of th r d blood o puscl *J p Med* *17* 324.

I THE ERYTHROCYTIC PARASITES



GENERAL STRUCTURE

II THE ERYTHROCYTIC PARASITES NUCLEAR STRUCTURE

The living nucleus is not clearly visible by ordinary methods of examination and for this reason *the nucleus of the malaria parasite* has been studied mainly in films which have been fixed and stained by special methods The ordinary methods of staining for malaria diagnosis give a general indication of the distribution of nuclear material, but they fail to reveal the fine details which may be seen in films which have been prepared and stained by methods appropriate for studies of nuclear structure

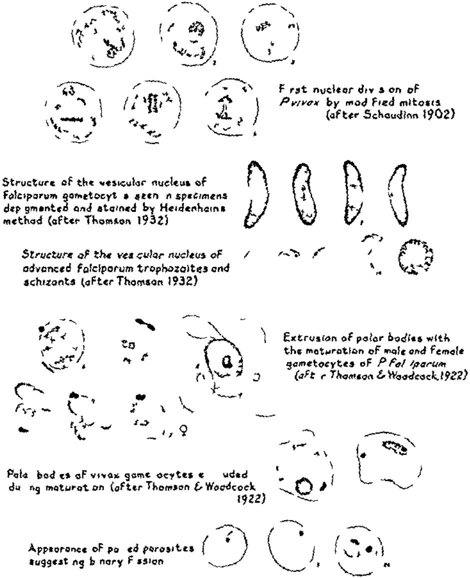
Structure—Seen in Romanowsky stained films the nuclei of the asexual parasites appear to be dense and structureless, the chromatin stains so deeply that the internal detail is lost But with haematoxylin staining Thomson *(1932) obtained appearances in the schizogonic forms of P falciparum* which led him to believe that the nuclei are vesicular with a limiting membrane and a fairly large karyosome (11 15) The gametocytes too seemed to have a vesicular type of nucleus with the chromatin distributed around the periphery and a nucleolar mass or karyosome in the centre (7 10) Wolcott (1954), on the other hand found no evidence of a vesicular *structure in the nuclei of vivax trophozoites, the resting nucleus appeared to consist essentially* of two coiled chromosomes with no limiting membrane

Division—The nucleus of the erythrocytic malaria parasite is thought to divide by a form of mitosis According to Schaudinn (1902) the process in *P vivax* begins as the chromatin granules collect as a band or equatorial plate across the nucleus, the band divides throughout its length and the nuclear material in the two halves migrates to the opposite poles to form two separate nuclei (1 6) The recent studies of Wolcott (1954) have shed more light on the process The two chromosomes of the vivax nucleus arrange themselves at the equator of a well formed achromatic spindle then they divide and move to the poles according to the usual pattern found in other organisms

Polar bodies—Mitotic division of the nuclei leaves the daughter nuclei with the same number of chromosomes as the parent nucleus The special form of division which halves the *number of chromosomes—a necessary reduction in the chromosome* complement of the sex cells if the progeny of the fertilised ovum are to have the normal number—is known as the reduction division Presumably there is some such special division during the sexual development of the malaria parasites and the appearance of nuclear material apparently discarded from the cell on the surface of the gametocytes during maturation the so-called polar bodies has been held to mean that a reduction division occurs at this stage Figures 16 23 taken from Thomson and Woodcock (1922) portray the appearance of the apparent polar bodies in the maturating gametocytes of *P falciparum* and *P vivax* The problem seemingly still unresolved is discussed by Ann Bishop in a recent paper

Binary fission—Binary fission is a primitive method of reproduction in which two *daughter organisms arise by a simple amitotic division of the parent* The body lengthens a constriction appears in the middle and the organism separates into two halves Whether the malaria parasites ever divide by binary fission is not definitely established Reproduction by binary fission has been held to explain a multiple infection of host cells to a degree which seems out of proportion to the number of parasites in the blood The rings, it is said may simply *divide into two, four* or even more Reproduction in this manner has also been held to explain the doubling of the nuclear chromatin in falciparum ring forms and the curious symmetry which is sometimes seen in adjacent rings Most workers in malaria however would probably agree that the evidence for binary fission in the malaria parasites is *far from conclusive*

SCHAUDINN F (902) C d by WENYON C. M (19 6)
WENYON C. M., *Pr rootogy V I 2* Bailliere Tindall and Cox Lond 926 p 9 7
WOLCOTT 1954 (f p 16) Thomson 1932 (ref p 16)
THOMSON AND WOODCOCK 1922 (ref. p 8)
BISHOP ANN (1955) Pr bl m oncerned with gam tog sis in Haemosporidiude w th particular refer ce t th Genus *Plasm di m P asit l gy 45* 63



NUCLEAR STRUCTURE

III THE ERYTHROCYTIC PARASITES POSITION IN THE HOST CELL

The primary relation of the malaria parasite to the red blood corpuscle is one of simple attachment Minute pseudopodia thrown out from the young trophozoite are thought to grip the cell surface, sometimes not very firmly for organisms which have become detached from the host cell are not uncommon Figures 9 and 10 show cells which, from the characteristic stippling have clearly been invaded by parasites but have lost them by detachment from the surface Mary Lawson (1919) believes that this phenomenon is by no means unusual The parasite she thinks, may invade and destroy several cells in succession flitting from cell to cell like a butterfly from flower to flower A degree of anaemia which seems out of proportion to the number of parasites in the blood may be due, she suggests, to this migratory and predatory habit Rarely the older parasites may be partly or wholly detached from the host cell, they may even lie free in the plasma Figures 11 and 12 portray vivax trophozoites which are partly outside the host cell, figure 13 represents an advanced falciparum trophozoite which is attached by delicate strands to the host cell, an interesting relationship which recalls the views of Maurer that the spots which now bear his name are the sites of attachment of the organism to the host cell (*see* also plate VIII, fig 2)

Does the malaria parasite enter the host cell at some stage in its growth, or does it always stay on the surface? Four possible relationships are illustrated in figures 5 8 We must accept the original surface attachment of parasites which have abandoned intact red cells heavily studded with Schuffner s dots or Maurer s spots (9 10), and we cannot doubt the extra cellular attachment of parasites like those portrayed in figures 11 13 The red cell apron or bib spanning the concavity of the crescent (17) would scarcely have this appearance, with the poles of the crescent projecting beyond the points of attachment of the bib were the crescent within the cell But appearances are deceptive and the lack of precise knowledge on the internal structure of the red cell itself adds a further element of uncertainty If the parasite lies on the cell surface why does it so seldom appear beyond the edge? Why does the mature organism filling the cell, appear to keep within its bounds? Is it confined within an enveloping red cell, or is it free to curl over the outside edge and pass to the under surface while still conforming to the approximate cell outline? Can it be that the parasites are both intra and extra-cellular that they erode into the host cells eating into their substance but that one surface of the parasites is external and in direct relation with the plasma? Against this theory are the observations of Ratcliffe (1927) that in thin sections of infected red cells the parasites appear to lie within the cells and the studies of the parasites under the electron microscope which have led Wolpers (1942) and Fulton and Flewitt (1955) to the view that the parasites grow under the shelter of the red cell membrane

ROWLEY LAWSON MARY (1914) A stage in the migration of the adult tertian malarial parasite Evidence of the extra-cellular location of the parasite to the red corpuscle *J Exp Med* 19 45

— — (1918) Aestivo-autumnal malaria. The extracellular location of the crescent bodies to the red corpuscle and their method of securing attachment. *J Exp Med* 27 739

— — (1919) Migration of parasite as the cause of anemia in aestivo-autumnal malarial infections *J Exp Med* 29 361

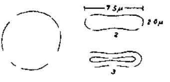
RATCLIFFE 1927 (ref p 21)

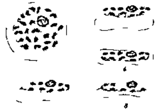
WOLPERS 1942 (ref p)

FULTON J D AND FLEWITT T H (1955) Electron microscope studies in malaria. *Trans R Soc trop Med Hyg* 49 30

III THE ERYTHROCYTIC PARASITES

The red blood corpuscle is a flexible bicon ave disc Has it a thin envelope containing haemoglobin enmeshed in a fine reticulum or does it consist mainly of a thick envelope?



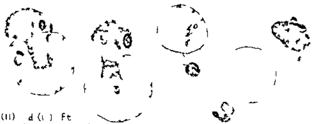


Does the mature organism lie on the surface of the cell or within the cell or both within and without covering an eros on eaten on the cell like a scab on an ulcer?

(5) Attach.d to the thin limiting membrane of a balloon like red cell?
(6) Within the l miting membrane of the cell?
(7) Eroding through a th n limiting membrane?
(8) Eroding through a thick red cell envelope?

Former host cells showing Maurers and Schuffners dots the parasites wh ch have left the cells were clearly extra corpuscular





(11) d (1) Ft
M y L w c 1914

Parasites which are partly or wholly free from the host cell

The remains of the red cell form an apron in the concavity of the crescent (16) would be the appearance of the apron were the parasite ntracorpuscular (17) is the normal appearance (after Mary Lawson 1918)



POSITION IN RELATION TO THE HOST CELL

Invasion of host cells—In Malayan infections the earliest invasion of the red blood corpuscles by *Plasmodium falciparum* is represented by parasites (2 4) which have no more than an incipient vacuole and are little larger than the merozoites (6) from which they arise These very young forms are unusual in blood films, invasion occurs mainly in the internal organs and the infected cells may not enter the peripheral circulation until the parasites are already several hours old The youngest forms normally found in blood films are considerably larger than merozoites, they have a well developed vacuole and typically a single or double bead of nuclear chromatin (5, 7, 8) The vacuole is sometimes paler than the rest of the host cell, a change possibly due to the removal of haemoglobin by the growing organism Sometimes the smallest forms seen in any particular infection are even larger (9 11) a difference which may be related to a longer retention of the infected cells in the internal capillaries The ruptured schizont portrayed as figure 6 gives an indication of the relative sizes of the merozoites and the early ring form trophozoites

Cytoplasm—The typical arrangement of the cytoplasm is the well known ring formation but variations in form are not uncommon with the cytoplasm disposed as an irregular mass adjacent to the nucleus (12 16) in delicate wisps and strands the so called tenue form (28, 29), or draped as a thin line along the margin of the cell (22 24)

Chromatin—The characteristic nucleus is a single minute bead of chromatin (7, 8) Doubling of the chromatin is common often with one bead smaller than the other (5 18 20) and trebled chromatin is not rare (12, 21 28, 30) * Sometimes two beads may appear to be fused together, looking rather like a diplococcus (9 26, 29) or the chromatin may take the form of short curved bar (11, 33) Usually the chromatin lies on the periphery of the ring projecting evenly within and without The apparent size of the bead varies even among parasites of the same stage of growth (cf 3 and 8), this variation may be adventitious produced during the spreading of the film or it may represent a real difference Despite these differences in the patterns of the chromatin in the young trophozoite, the range of variation is less than at later stages

Pigment—No pigment is visible at this stage

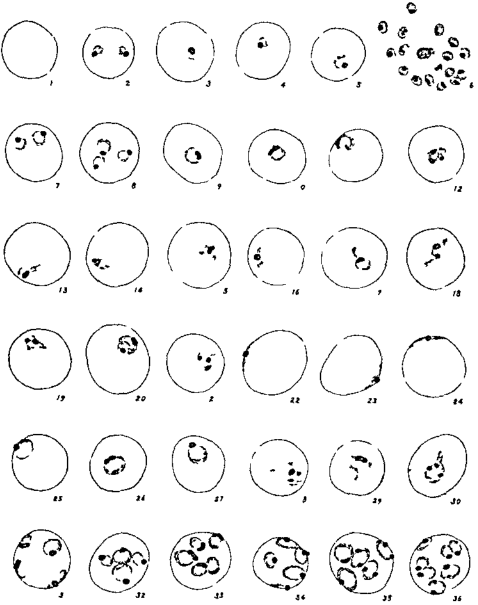
Stippling—Maurer s spots are not normally seen at this stage a few early spots are shown in figures 30 and 34

Other changes in host cells—No obvious changes in the host cells are visible in stained films at this stage The marked shrinkage in the size of the infected cell described by the early Italian workers, seldom occurs in cells containing the Malayan parasites The variations in size of the host cells reflect the anisocytosis which is not uncommon in falciparum infections as in all malaria infections

Multiple infections of host cells—The invasion of single cells by more than one parasite is a common occurrence in falciparum infections Two three four or more parasites may be crowded into a single cell (31 36), eight rings in a cell has been recorded The cell shown as figure 36 was drawn from a film taken just before death from a patient with a parasite density of more than one million per c mm blood The incidence of multiple infected cells in this patient agreed fairly closely with the mathematical odds of random distribution (*see also* p 30)

* This curious doubling of the chromatin bead has been interpreted (*a*) as fragmentation of tenuous strands of chromatin during contraction of the organism to the ring form in the drying films (*b*) as premature division of the nucleus and (*c*) as an expression of phylogenetic relationship between malaria parasites and blood flagellates We regard the first explanation as the most likely

IV *Plasmodium falciparum*



EARLY TROPHOZOITES

V *Plasmodium falciparum* MARGINAL TROPHOZOITES

The young trophozoites of *Plasmodium falciparum* are often draped around the edge of the host cell, a marginal position seldom assumed by other species These marginal forms also called accole or appliqué forms, are sometimes a guide to species diagnosis They are also a common cause of missed diagnosis for the young marginal parasite, a mere thickened line and a chromatin bead along the cell margin, is easily overlooked (2 5) Marginal parasites are not found in all or even in most falciparum infections, when they are present they are likely to be in a minority, sometimes they outnumber all other forms

Form—The marginal forms of *P falciparum* have three characteristic patterns which for ease of description we may call the common, the rim and the 'displaced forms The common form is shown in figures 13 to 16 The single chromatin bead lies on the edge of the cell, with most of cytoplasm extended along the edge on both sides of the bead the parasite overlaps the edge and a faint cytoplasmic line, barely visible on the surface of the cell completes the appearance of a flattened ring The chromatin may be doubled (10 11, 17, 18), and there may be considerable cytoplasmic irregularity (11 12, 20) These flattened marginal rings and also the displaced forms described below, were clearly portrayed by Maurer (1902) in the Sumatran parasite

The 'rim formation is illustrated in figures 2 6 The complete parasite lies like a thickened line along the rim of the host cell, with no evidence of a ring formation (2 5) We cannot too strongly emphasise the importance of these forms as a potential source of diagnostic error Recognition depends on the identification of nuclear chromatin and on the relation of the chromatin to the marginal line of cytoplasm

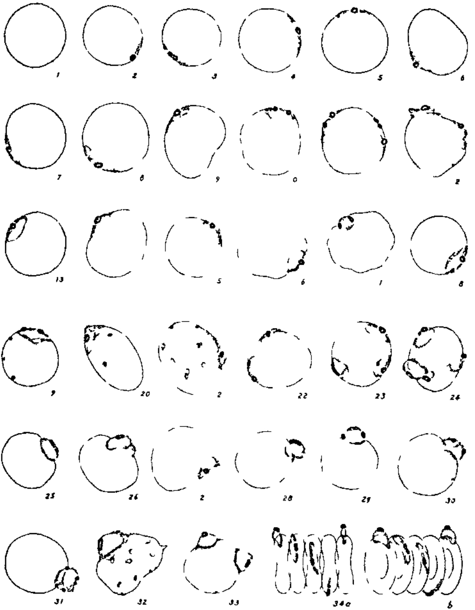
Marginal parasites which are displaced beyond the edge of the host cell are fairly common (25 33) All degrees of displacement may occur, from a partial displacement with the parasite indenting the edge of the cell (25, 26) to a marked displacement with most of the parasite lying beyond the cell margin (31 33) A ring formation often distorted is usual The chromatin may appear to lie on the cell margin (31), but more often it appears on the periphery of the ring some distance from the cell (25, 30 33) The form of the nuclear chromatin may be that of a single bead (26 27, 33) a double bead (29, 30), or a curved bar (25 28) Three chromatin beads possibly four are visible in the parasite portrayed in figure 31

Age—Garnham (1933) suggests that an accole phase may appear late in the cycle when the parasites are about 24 hours old We have seen no evidence of such a phase in the Malayan organism Early trophozoites (2 6), and advanced trophozoites in cells stippled with Maurer s spots (20 21, 32), may alike have a marginal position

Cause—The marginal position of *P falciparum* in particular infections is unrelated to technique Repeated films are likely to show the same phenomenon In the film from which some of the figures were drawn nearly 80 per cent (389/500) of the parasites were marginal twenty four hours later 74 per cent of the parasites still had a marginal attachment the infection was mild with a parasite density of 11,000 per c mm of blood Why is it that *P falciparum* often invades the margin of the host cell while other species rarely do so? We are inclined to seek the cause in the particular conditions under which invasion occurs in the internal capillaries A speculative explanation is suggested in figures 34a and b Erythrocytes circulating in rouleaux formation would present only their edges to the invading parasites a marginal attachment of the parasites would be a logical consequence Aggregations of erythrocytes have been observed *in vivo* by capillary microscopy (Wintrobe, 1946) Do these aggregations take the form of rouleaux? Do they occur and are they particularly common in falciparum malaria? We do not know

MAURER 1902 (f p 10)
GARNHAM P C C (933) A n t n th ubt rti malaria para e of Ke ya *E Af m d J 10* 68
WINTROBE M M *Cl ni l H em t logy* Henry Kimpt Lo d 1946

V *Plasmodium falciparum*



MARGINAL TROPHOZOITES

VI *Plasmodium falciparum* ADVANCED TROPHOZOITES

The parasites we describe as advanced trophozoites are at the stage of development which begins with the appearance of pigment in the cytoplasm and of Maurer s spots in the host cells, and ends *with the first division of the nuclear chromatin* *Trophozoites* at the stage of growth portrayed in figures 2 24 are likely to be found in blood films, taken at the appropriate stage of the attack, in most falciparum infections of Malayan origin Their presence in the peripheral blood does not seem to be related as on parts of Africa, to immunity in the host (*see* also p 26) *The later stages (26 30), leading up to nuclear division are seldom seen in* blood films except in severe infections

Form—The typical forms in blood films (2 24) are fleshy, rounded or ovoid rings, with abundant cytoplasm a single mass of chromatin, and a vacuole which sometimes has a marked pallor Morphological variations are common particularly in the nuclear chromatin The later forms (26-30) have little or no vacuole The final stages with imminent nuclear division are shown in figures 29 30

Cytoplasm—The *ring form is usual in the earlier forms shown in the plate (2 12)* but with advancing growth the ring appearance is lost and the cytoplasm encroaches on the vacuole (14, 16 24) With the loss of the vacuole, the parasites appear to contract, and the later forms are little bigger than the more advanced rings sometimes indeed, they are smaller (26-28)

Chromatin—The nuclear chromatin of the advanced trophozoite is still characteristically a single bead but it may be doubled (7 17, 18) or curvilinear (14 16) Sited typically on the periphery of the ring it may lie just within the periphery (3 8, 11) or even within the vacuole (5 17 24) A common form is that of a curved bar aligned along the inner wall of the vacuole (14 16) With the approach of nuclear division, the chromatin mass becomes larger and more diffuse (27 30)

Pigment—A few minute granules of pigment, invisible in the stained parasite appear in the cytoplasm at the beginning of this stage The granules increase in size and number until they become visible in the stained film as a greenish yellow tinge in the cytoplasm At first discrete the granules begin to concentrate and to coalesce before the onset of nuclear division already in the presegmenter portrayed as figure 30 the pigment has fused to a single mass (*see* also plate XXXI)

Maurer s spots—Maurer s spots beginning as a few minute dots in the host cell (2 4) are seen in their most characteristic form in cells containing large trophozoites which have not yet lost their vacuole (8 24) At a later stage the spots may still be typical (27), or more numerous *and less distinctive (26)*

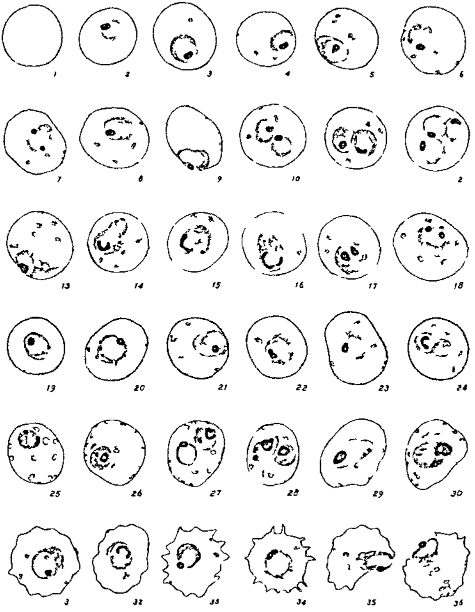
Darkening of the infected cell—A purplish tinge in the red cells containing large trophozoites, described by Maurer (190_) and illustrated by Thomson (1924), is not uncommon The affected cells often have a darkened rim (20-24) a change also recognised by Maurer (*see* also plate XXXVII *fig 4*)

Crenated host cells—A spiky outline is common in red cells which have dried slowly Cells infected by *P falciparum* are peculiarly liable to this distortion of outline (31 36) Marchiafava and Bignami (1894) and Mannaberg (1894) portray this appearance in host cells which are shrunken in size and darkened in colour

Size of host cells—There is no significant change in the size of infected cells

MAURER, 19 2 (f p) Th ms n 924 (f p 9) Marchiaf a and B gnami 1894 (ref. p 3) Mannabe g 894 (ref p 5)

VI *Plasmodium falciparum*



ADVANCED TROPHOZOITES

The typical nucleus of the falciparum trophozoite in the Romanowsky stained thin blood film has the appearance of a dense, deeply stained circular bead This may be regarded as a resting phase In life the organism is in constant movement with the nucleus incessantly changing in form with the streaming flow of the cytoplasm and we may conceive that any sudden arrest of this movement, by fixation or quick drying, would tend to perpetuate the bizarre forms of the active nucleus We may further suppose that the active nucleus, stretched maybe in flimsy strands is peculiarly liable to damage when, exposed to the physical stresses of dessication or fixation, it begins to contract to the resting state We may thus assume that the varied forms of the nuclear chromatin express (*a*) the sudden arrest of the movements of the living organism as the films dry, and (*b*) the fragmentation of the nucleus as it contracts against resistance to the resting state

Size—The size of the trophozoite nucleus varies of course with the stage of growth of the organism but there is variation also in the apparent size of the nuclei in trophozoites at the same phase (2, 4) Chromatin beads which are usually large in relation to the size of the rings are not uncommon we suspect that this appearance may sometimes be adventitious as large beads may appear in a single film with beads of normal size in other films from the same case but real differences in the amount of nuclear chromatin undoubtedly occur

Nuclear bead—The characteristic nucleus in the young trophozoite appears as a round or ovoid bead placed on the periphery of the ring usually where the ring is thinnest (2, 3) The bead as a rule, is single but double beads are very common Variations from this basic pattern occur mainly at later stages when the amount of nuclear chromatin is greater

Doubling of the nuclear bead—A single ring often has two beads of nuclear chromatin (4, 5), sometimes three (21, 22) This tendency, from its comparative frequency in *P falciparum* is sometimes useful in diagnosis The beads may be side by side (12) at opposite sides of the ring (4) or elsewhere on the ring (5) One bead may be much smaller than the other (4, 5) or the two may have the same size (20) The cause of this doubling of the chromatin is unknown.

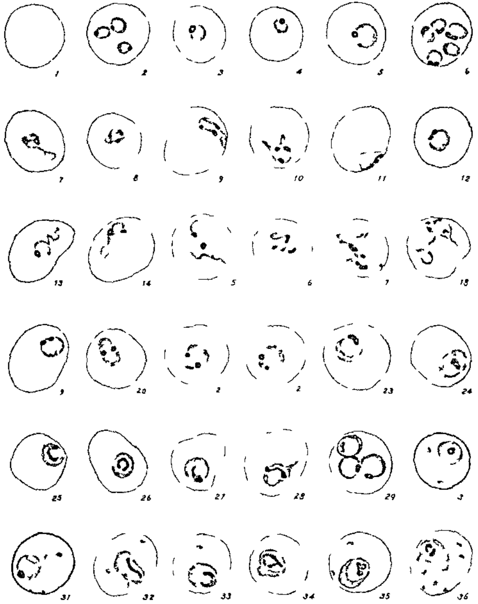
Linear nuclei—A common nuclear pattern particularly in the older trophozoites is the curvilinear form illustrated with several examples in the plate The nucleus may have the appearance of a short curved bar (6, 23) a half circle (25, 33) or even a circle nearly complete (26), or the curves may take bizarre shapes (28, 32, 34) The long thin streaks or lines of chromatin which may appear in the band forms of *P falciparum* are illustrated in plate IX.

Bizarre nuclei—Nuclear patterns of great variety, with all degrees of distortion or fragmentation, are common in the tenue forms of *P falciparum* The plate gives a few examples (16–18), others are shown in plate XIX We may perhaps speculate whether some of these appearances are to be interpreted in terms of basic nuclear structure Wolcott (1954) has shown that the non dividing nuclei of one species of Plasmodium—*P vivax*—consists essentially of two coiled and condensed chromosomes

Position—Sited as a rule on the circumference of the ring the nuclear chromatin may also lie just within the periphery (23 29), or even in the centre of the vacuole (30, 36) depending perhaps on the orientation of the parasite in relation to the cell surface Nuclei which have a curvilinear form often appear to lie just within the vacuole, curled around the inner wall (26 32 33) Ring form trophozoites with a central bead of chromatin have been aptly described as bird s eye forms (30) This bird s-eye appearance is somewhat unusual in falciparum trophozoites but very common in the trophozoites of *P malariae*

WOLCOTT 1954 (ref. p 16)

VII *Plasmodium falciparum*



VARIATIONS IN THE NUCLEAR CHROMATIN OF THE TROPHOZOITES

VIII Plasmodium falciparum MAURER S SPOTS

The coarse stippling in red blood cells infected by *Plasmodium falciparum*, now known as Maurer s spots, was first portrayed by Schuffner (1899) whose original drawings are reproduced as figures 10-12 Maurer s detailed account in 1902 was accompanied by the illustrations which have been re drawn as figures 1 9

Maurer s spots may be described according to their stage as early', developed , and late , distinctions which emphasise the marked change in their appearance as the schizogonic cycle of the parasite unfolds

Early—Maurer s spots are slow to appear Cells containing young falciparum rings are free from stippling (13), unlike vivax infected cells which are heavily stippled within a few hours of invasion The first evidence of stippling is normally seen when the parasites have reached the stage shown in figures 14 or 15, these particular cells are unstippled indicating the variation which may occur The first stipples are minute purplish points , half a dozen or less in number (16 18), the points become dots , still few in number and are now characteristic enough to be recognised (19 20)

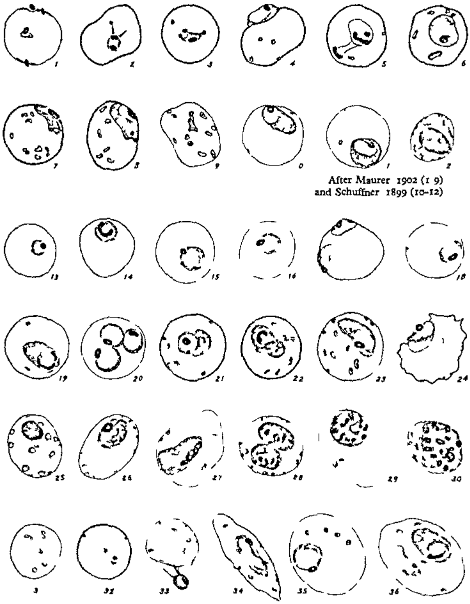
Developed—Maurer s spots are seen in their most characteristic form in cells containing large falciparum rings (21 24) They are always few in number at this stage and, as Maurer remarks, easy to count Maurer describes them as fine ringlets (feinste Ringelchen), loops (Schleifen) or streaks (Streifen) To this description we would merely add the appearance of irregular blotches which some of the spots assume The characteristic difference between the stippling in falciparum infected and vivax infected cells is implicit in the terms Fleckung (flecks or spots) and Tupfelung (dots) used by Schuffner and in this account we have followed James (1929) in referring to the stipples in the falciparum infected cell as spots and to the stipples of the vivax infected cells as dots In well stained blood films from patients with falciparum infections of Malayan origin, Maurer s spots are seldom absent from the red cells containing large rings but the staining of the spots is very sensitive to pH they stain best at a pH of 7 2 7 6, and can seldom be demonstrated if the pH falls below 6 8 (*see* also plate XXXVIII)

Late—As the host cell becomes degenerate Maurer's spots tend to lose their characteristic appearance The stipples become more numerous and less regular, and sometimes, instead of discrete spots, there remains but a diffuse vague purplish reticuluum This change in the character of the stippling tends to begin at about the time of the first nuclear division of the parasite and for this reason is seldom seen in blood films Figures 26 30 portray the changed appearance of the spots in cells containing presegmenters (26 28) and schizonts (29 30) A point of minor historical interest is that this late stippling so unlike Maurer's spots was clearly illustrated by Schuffner (1899) whose drawing is reproduced as figure 1ۦ

Various—Cells with characteristic Maurer s spots but with no parasite are not uncommon The stage of the spots in the cells drawn in figures 9 31 32 indicate that the parasites were at least twenty four hours old when they were detached from the cell figure 32 also shows the thickening of the cell margin which is an occasional characteristic of the falciparum infected cell while figure 33 shows the parasite still attached These appearances suggest that the organism was extracellular at the time of detachment Maurer s spots in a distorted host cell are portrayed in figure 34 figures 35 and 36 show developed and distorted Maurer s stippling in macrocytic erythrocytes

SCHÜFFNER 1899 (cf p 10) Maurer 1902 (cf p 10)

VIII *Plasmodium falciparum*



After Maurer 1902 (1 9)
and Schuffner 1899 (10-12)

MAURER S SPOTS

IX. *Plasmodium falciparum* BAND AND OTHER BIZARRE FORMS

Band forms—Malaria parasites which stretch across the host cell like a band have long been known This band formation is particularly common in the trophozoites of *P malariae* Less well known on account of their comparative rarity and diagnostic irrelevance are the band forms which occur in falciparum infections Among the first to illustrate the band forms of *P falciparum* with many examples, was Mary Lawson (1911) who portrays young trophozoites drawn out all in the same direction as slender lines across the host cells, a distortion apparently produced by spreading the blood as a film (13 15) Sergent *et al* (1913), finding the same forms in blood films from a child with comatose malaria mentions their similarity to the trophozoites of *P malariae* (16-18) Callanan (1926) shows a similar distortion of the older falciparum trophozoites, again in the same direction seemingly produced by a rolling of the host cells between slide and spreader in the preparation of films Band forms in *P falciparum* of Malayan origin are illustrated in figures 1 12 The parasites may be very young (4) or so advanced in growth that Maurer s dots are present in the host cells (7 12) band schizonts are not unknown (*see* plate XI) The cytoplasm may stretch across the cell as a linear streak (4 5), or appear as a narrow drawn out ring (2 3, 8) As a rule the nuclear chromatin is similarly distorted into a single line along the band (1, 2 5), but it may persist as an ovoid bead (9, 12) Sometimes the host cell is elongated in the same direction as the band (12)

In Malayan infections the conditions under which falciparum band forms are liable to appear are fairly well defined The infections are usually heavy The bands appear at the end of the spread where the blood is spread thin, they occur in groups and are usually distorted in the *same direction Brug (1934) and Sinton (1934) have discussed the cause of the phenomenon* The bands are found in parts of the film where the blood dries quickly, where distortions of the parasites either natural from their amoeboid activity or artificial from physical stresses during the spread of the blood, would be fixed before the organisms could resume their normal or resting form

Symmetrical rings—Trophozoites which have a curious symmetry have been described by Stephens (1924) in a falciparum infection of West African origin Adjacent parasites in double infected cells appeared almost as mirror images (20-23 25) double nuclei *in a single* parasite sometimes displayed a like symmetry (24) The explanation is unknown

Parasites devoid of cytoplasm or chromatin—Macfie and Ingram (1917), working in the Gold Coast have *described red cell inclusions which they believed to be abnormal* malaria parasites in patients taking prophylactic quinine They portray chromatin masses without cytoplasm (27 29) and cytoplasmic rings without chromatin (30 31) If the forms illustrated in figures 27 29 are indeed malaria parasites, they suggest either a marked damage by quinine, o a failure to stain the cytoplasm from the use of too acid a stain Malaria diagnosis on such evidence has obvious pitfalls No red cell inclusion should be regarded as a young malaria parasite unless it has both cytoplasm and nuclear chromatin This arbitrary working rule is a necessary safeguard *from error*

ROWLEY LAWSON M. Y (9) Th tu o- utumnal para it t exual cy l in th ci cul ting blood of man w th d scrip- ti n f th m rph l gical and b ological char cter tics f th paras e *J xp M d 13 263*

SERGENT EDM SERGENT ET B GUET M AND PLANTIER A (1913) Ob ervations microscopiques u cours d un acces p rnici u pal dé n. *Bull S P t ot 6* 615

CALLANAN J C. J (19 6) A d light on th p od ct n f *Plasm d m tenue* (Stephens) *Keny med J 3* 82

B UG S L. (934) Ob rv ti n monkey malaria *R M lan J 13* Sea 1 1

S NTON J A (1934) A q arta malaria para f th low r on ntal m nk y *S lenus rus* (*M cus cynomolgus*) *Rec Mal S rv I d 4* 379

STEPHENS J W W (1924) S m m rph l gical f tur f *Plasm dium f lciparum Ann tr p M d P asit 18* 33

MACFIE J W S AND INGRAM A (9 7) Ob rv tions o malaria in the Gold Co t Colony West Africa *Ann tr p Med P nt 11*

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ROWLEY LAWSON MARY (1911) Th ae u o- utumnal para t cs exual cycle *in th circul* u *g blood* of man with a d crip u n of th m rphological d b ol g cal char cte tics f th p as *J xp M d 13* 263

SERGENT EDM S GENT ET B GUET M AND PLANTIER A (19 3) Ob erv u microscop qu s cours d un cè p rni eu paludé *B ll S P t t 6 6 5*

CALLANAN J C J (19 6) A de light the p od ctı n of *Plasmod um tenue* (Steph s) *Keny m d J 3* 82

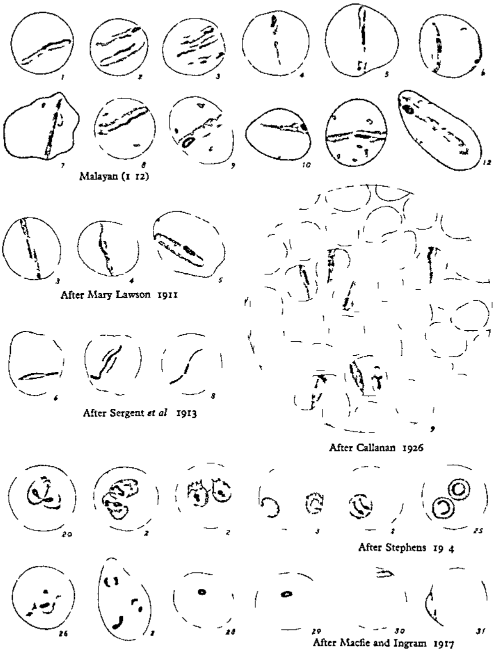
BRUG S L (1934) Ob rv u n n mo k y mal ria *R v M l ri I 13 Se 1* 121

SINTON J A (934) A quartan malari p te of the l w ori tal monk y *S len rus* (*M cu cynomolgus*) *R Mal S rv Ind 4* 379

STEPHEN J W W (1924) S m m rph l gi al featur f *Plasmod um f l p rum Ann t p M d P ast 18* 33

MACF J W S AND INGRAM A (917) Ob rv t ns n mal ria in th Gold Co t Colony West Africa *Ann t p Med P 11* 1

IX *Plasmodium falciparum*



BAND AND OTHER BIZARRE FORMS

X *Plasmodium falciparum* SCHIZONTS

Definition—Malaria schizonts in a strict zoological sense are the asexual erythrocytic parasites at all stages of the schizogonic cycle, regardless of the state of the nucleus The term is used here to describe the parasites at a stage of active or completed nuclear division

Occurrence in the peripheral blood—In falciparum infections of Malayan origin schizonts seldom reach the peripheral blood except in heavy infections In the large series of cases, schizonts were seen in blood films in two per cent of mild infections but in sixty per cent of heavy infections, young schizonts (2 12) were more common in blood films than advanced or mature schizonts (19 36)

Form—The schizonts are compact rounded bodies They have no vacuole Occupying about one third of the host cell at the time of the first nuclear division, they take up two thirds or more of the cell when nuclear division is complete (22 23 25) or they may fill the entire cell (28) Welch (1897) states that the mature schizonts take up only a relatively small part of the host cell, schizonts so small are unusual in Malayan infections Garnham (1933) had described schizonts which have a crescentic form, they occur in the Kenya parasites, mainly in cultures and rarely in the peripheral blood We have not seen these forms in the Malayan organism

Cytoplasm—The cytoplasm is fairly dense and often stained so deeply that the chromatin masses from the first nuclear division are not easily seen It remains fairly homogenous until its final separation to invest the individual merozoites (34 36)

Nuclear chromatin—Often loose and ill defined at the beginning of schizogony, the nuclear segments appear as vividly stained purple ovoids in the later stages The segments are somewhat irregular in shape and size in the early stages of nuclear division (7 18) but in the mature parasite they are fairly uniform (27 30)

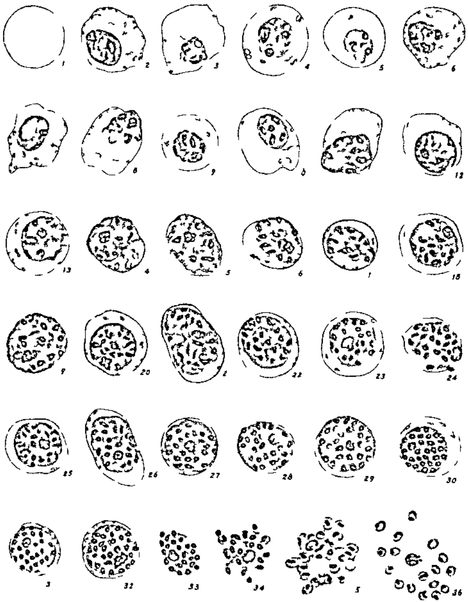
Pigment—The pigment of the schizont is typically fused into a single rounded mass Beginning with a concentration of the granules at the presegmenting stage of the parasite, this fusion of the pigment to a single—sometimes a double—mass is usually advanced at the first nuclear division The pigment mass may occupy almost any position in the parasite central (27 29) eccentric (26 32) or peripheral (18 24) The colour of the pigment varies with the optical conditions of examination, usually a dark mahogany brown it may appear almost black with poor illumination

Merozoites—The plate illustrates merozoites which are still clustered together (33) or scattered from the bursting of the ripe segmenter (34 36) Typically, the merozoites are round or ovoid beads of chromatin eccentrically placed in the enveloping cytoplasm They are usually compact, though the beginnings of a vacuole are sometimes evident (35) The number of merozoites in a single schizont varies in Malayan infections from 15 or less to 30 or more, with an average of about 20, extremely wide variations from less than ten to more than forty have been reported from various parts of the world Peel and van Hoof (1948) in the Belgian Congo found an average of 30 merozoites in schizonts from the placenta, but 20 only in schizonts from the peripheral blood

Changes in host cell—The variations in size of the host cells are no greater than the variations in the uninfected cells Maurer s spots, though still distinctive in some of the cells containing early schizonts (5 9) tend to increase in number and to lose their characteristic features as the parasite grows and the host cell degenerates (6 8 10-12)

GARNHAM 1933 (ref p 29) Welch 1897 (ref p 46) Peel and V H f 1948 (ef p 85)

X *Plasmodium falciparum*



SCHIZONTS

XI *Plasmodium falciparum* UNUSUAL OR BIZARRE SCHIZONTS

Schizonts of *Plasmodium falciparum* have been described which differ so much in their morphological characters from the usual forms that they have sometimes been regarded as new species or varieties Plate XI illustrates some of these abnormal forms

Small schizonts—The schizonts of the quotidian variety of *P falciparum* recognised in Italy by Marchiafava and Bignami (1894), were excessively small (figs 2 6) The same quotidian variety was recognised by Craig (1909) in the Philippines figures 7 9 have been redrawn from his photomicrographs (Craig, 1921) The schizonts portrayed by Marchiafava and Bignami are much smaller than any we recall having seen in Malaya but we are inclined to think that the size of the schizonts described by Craig falls within the range of variation of the Malayan organism (cf plate X) Thomson (1914) has shown how quinine treatment may influence the appearance of the schizonts (10-12)

Bizarre nuclei—Unusual appearances in the form of the nuclear chromatin are portrayed by Bastianelli and Bignami (1899) and by Jolly (1936) Some of these appearances we do not recall having seen in the Malayan organism—the clusters of chromatin dots in the young schizont (15) the appearance of paired linear fragments of chromatin (16), the coarse curvilinear form of the chromatin segments (22) and the curious V forms shown in fig 23

Absence of pigment—Welch (1897), in his description of *P falciparum* refers to schizonts which are devoid of pigment particularly in tropical strains and Mannaberg (1894) illustrates a cerebral capillary packed with schizonts which appear to be pigment free (14) Jerace (1939) records the presence of an unpigmented schizont in a normoblast (13) We have never seen unpigmented schizonts in the Malayan parasite

Scattered pigment—The pigment of falciparum schizonts is normally fused to a single mass at an early stage of nuclear division Jolly (1936) has described schizonts in which the pigment remains scattered at an advanced stage in schizogony (21, 23 24) We have not seen this appearance in the Malayan organism

Various—Falciparum schizonts which are unusual in their form or associations are portrayed in figures 27 38 drawn from Malayan material Schizonts which have a band form a rare appearance are shown in figures 29 and 30 and schizonts which share a cell with parasites of a different age or phase in figures 31-38 Various combinations are represented—schizont and schizont (36 38) schizont with ring form (33 35), and schizont with young gametocyte (32) Young schizonts in macrocytes are portrayed in figures 27 and 28 Maurer s spots of late phase numerous and somewhat resembling Schuffner s dots are well shown in figure 36

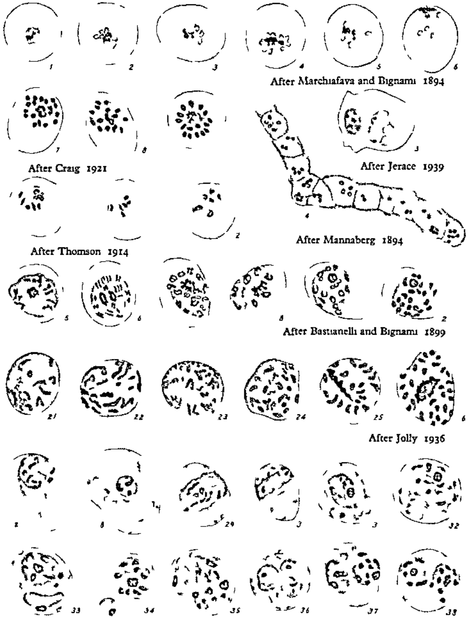
MARCHIAFAVA AND BIGNAMI 1894 (ref p 3) Craig 1921 (ref p 51) Mannaberg 1894 (ref p 5) Jolly 1936 (ref p 28)

JERACE F (1939) *R Malar* 18 Sez 1 233

THOMSON, D (1914) *The origin and development of gametes (crescents) in malignant tertian malaria Some observations on flagellation, etc* *Ann trop Med Parasit* 8 85

BASTIANELLI AND BIGNAMI 1899 (ref p 49)

XI *Plasmodium falciparum*



UNUSUAL OR BIZARRE SCHIZONTS

XII *Plasmodium falciparum* DEVELOPMENT OF THE GAMETOCYTES

The gametocytes of *Plasmodium falciparum* the familiar crescents do not normally develop in the peripheral blood Their nurseries are the capillaries of the bone marrow and spleen and only in severe infections are the early forms seen in blood films

Golgi—An early account of the developing crescentic bodies is given by Golgi (1885) who seems to have recognised only the later stages (1 7)

Marchiafava and Bignami—A more complete account is that of Marchiafava and Bignami (1894) who, working with preparations of the bone marrow and spleen, portray most of the developmental stages we recognise today (8 14), though necessarily without the refinements which came with the later advances in staining technique

Watson—The first account of the developing gametocytes of the Malayan parasite is that of Watson (1903) who recognised the forms described by Marchiafava and Bignami, and also portrayed minute rounded forms, with one or two grains of pigment which he regarded as the earliest stage (15 16) The patient was a Chinese man who died from a severe falciparum infection in Klang Hospital, twenty miles from Kuala Lumpur An account of the case was presented and films were exhibited at a Medical Congress held in Kuala Lumpur on 19th November 1902

Aragao—The sex of the young gametocytes is not easily determined Aragao (1930) attempts to define the differences of sex in the young forms found in the bone marrow The early male forms, he suggests, are intracellular rounded or ovoid bodies (22 24), while the females in their early stages are band like (28 29) or spindle-shaped (30 31)

Thomson and Robertson—In a fine colour plate Thomson and Robertson (1935) illustrate the form of the young gametocytes found in blood films from Malaysia, India and Africa (34 45) They depict most of the forms recognised by earlier observers—small intracellular, rounded or oat shaped forms (34 39) recognised by Marchiafava and Bignami (1894) and by Watson (1903), the spindle shaped forms (41 45) to which Laveran (1881) Swellengrebel (1913) Thomson (1914) Ioff (1930) and others, had drawn attention and the curious bodies in the red cells containing young gametocytes which Garnham (1933) had described (plate XIV)

A new conception of the development of the crescent—Brumpt (1952) has interpreted appearances of the parasites seen in the peripheral blood and bone marrow of a Vietnamese woman with a severe falciparum infection to mean that the early development of the gametocytes takes a course very different from that outlined in figures 1 45 Certain specialised parasites pass through two nuclear divisions to produce four elongated bodies arranged in parallel formation in the same cell These bodies he regards as the pregametocytes The forms from which the pregametocytes arise are the gamonts The appearance of the gamonts is described but not illustrated No supporting evidence for this interesting theory is known to us

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GOLGI (1885) Figs 7 dr wn fr m Giovann la 1938 (cf p 5)

MARCHIAFAVA AND BIGNAMI 894 (cf p 3)

WATSON M (1903) A n te on th para t of a case f malignant malaria, w th di cu s on n the d l pment of the crescent *J tr p M d (Hyg) 6* 221

ARAGAO H DE B (930) *Mem I t O w Cru 24* 41 Figure 22 33 edr wn f m G nnola, 1938 (f p 50)

THOMSON J G AND ROBERTSON A. (1935) The structure and dev lopment of *Plasmod um f l parum* game ocytes in th int rnal gans and peripheral circulation *T ans R Soc trop M d Hyg 29* 31

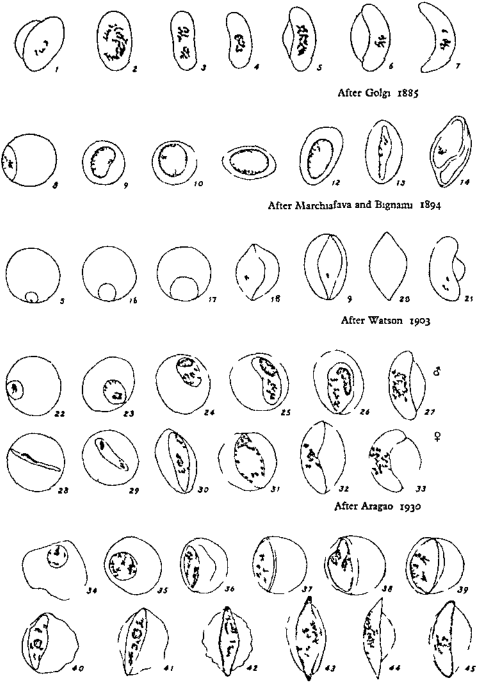
LAVERAN 188 (cf p 2) Th mson 9 4 (f p 138) Ioff 1930 (f p 48)

SWELLENGREBEL, N H (19 3) Schizogonie der w blich n Gam tocyten o *Laveran m l ria* (Trop ca—Paras t) *Zbl Bakt* Abt Orig *0* 179

GARNHAM P C C. (1933) On om curious bodies found i rythrocytes containing mmature cresc ts *T ns R Soc trop M d Hyg 26* 4 1

BRUMPT L C (95) Les gamo des pla modium humain *P v* et *P f l p rum B ll Soc P t t 45* 734

XII *Plasmodium falciparum*



THE DEVELOPMENT OF THE GAMETOCYTES

XIII *Plasmodium falciparum* EARLY GAMETOCYTES

Form—The earliest gametocytes of *Plasmodium falciparum* are rounded bodies with scattered grains of pigment and no vacuole (2 6) They lengthen with growth to become oat shaped (7 18) oval (19 22), cigar shaped (23, 24) or spindle shaped (27 34) The differences of shape, according to Aragao (1930) are determined by sex, the young males are rounded the females band like or spindle like The view of Aragao lacks confirmation, and in this account we make no attempt to differentiate the young male gametocytes from the young females

Intracellular rounded forms*—Watson (1903) states that the falciparum gametocyte can be recognised at a very early stage as a round pale staining body about the size of a small falciparum ring with a grain or two of pigment but without the characteristic vacuole of the asexual parasite (plate XII, 15, 16) When the parasite occupies about one third of the cell (2 3) the differentiation is more definite The cytoplasm stains a pale blue grey the chromatin is diffuse and not easily defined the pigment is scattered as small grains throughout the cytoplasm The later forms are still more characteristic They are larger rounded bodies with pale cytoplasm diffuse chromatin and numerous scattered granules of pigment (4 6) Garnham (1931) has drawn attention to the deep-staining peripheral condensation of protoplasm which gives the appearance of a capsule

Intracellular oat shaped forms*—These highly characteristic forms are portrayed by Watson (1903) and more clearly in the colour plate of Swellengrebel (1913) The parasite has roughly the shape of an oat grain (7 18) with pointed ends often with one side straighter than the other (8 11) The cytoplasm is rather pale, the chromatin is diffuse and often arranged along the straighter border, the pigment is scattered but not uniformly As in the earlier rounded forms the periphery of the parasite tends to stain more deeply than the centre, suggesting the presence of a capsule

Spindle forms—With further growth the gametocytes become elliptical (19 26) or spindle like with pointed ends (27 34) The appearance of a deeply stained periplast quite distinct from the red cell is not uncommon (31 34) The chromatin may appear as a reddish mass near the centre or it may be diffuse and difficult to define The pigment is scattered as granules or rodlets throughout the cytoplasm with a tendency to collect in the middle rather than at the poles The parasites may still lie within the bounds of the host cell (20-24), they may be attached to the remains of the host cell (31 34) or they may lie apparently free (26 30)

Changes in host cell—At first the host cells are unchanged in appearance later they tend to be stretched and distorted, and finally they remain only as tattered remnants adherent to the parasite Maurer s spots do not appear at any stage The inclusion bodies described by Garnham (1933) are not uncommon in cells containing the younger gametocytes (plate XIV) Host cells containing more than one parasite are portrayed in figures 35 and 36

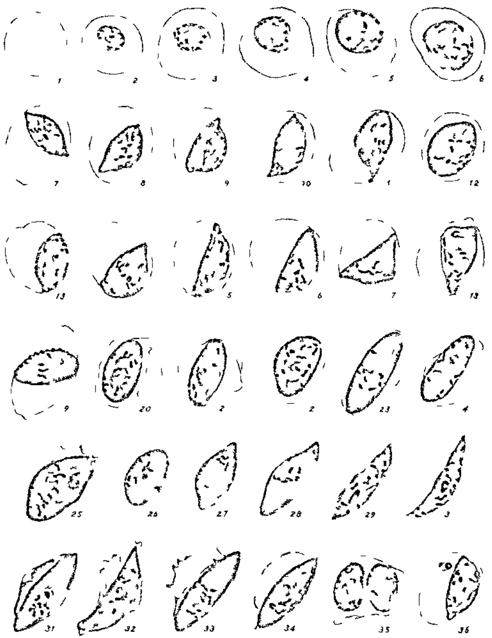
Association with heavy infection—These early gametocytes normally develop within the bone marrow or spleen, they appear in blood film only in serious infections The young intracellular circular and oat shaped gametocytes were drawn from films taken from four patients with a parasite density between 150 000 and 1 080 000 per c mm of blood two of these patients died from the infection

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* We use th term n racellular t m that the pa t ppear to h within th bound of the host cells with n implica on that th y t th b tance f th lls A this tag and ind ed t all tages w beli th gametocytes to be extra-corpuscular

ARAGAO 1930 (ref p 4) WATSON 1903 (f p 4) Garnham, 1931 (ref p 36) Garnham 1933 (f p 14) Sw ll greb l 19 3 (r f p 14)

XIII *Plasmodium falciparum*



EARLY GAMETOCYTES

XIV *Plasmodium falciparum* GARNHAM BODIES

Garnham (1935) has described inclusion bodies in the host cells of young falciparum gametocytes of East African origin The same bodies were demonstrated by Thomson (1933) in blood films from Sarawak, and portrayed in a colour plate by Thomson and Robertson (1935) They are not uncommon in falciparum infections of Malayan origin

Form—Garnham describes the bodies in these terms

i there is only one to a cell,

ii they consist primarily of a thick bar which stains a dark red colour,

iii they are usually one third to one half of the red cell in length but sometimes longer,

iv they usually lie free but sometimes seem to be attached to one end of the crescent,

v they may be curved to form loops the ends of which may join to form an ellipse or circle, or they may have a coil appearance,

vi the original bar may break up into fragments

The appearance of Garnham bodies in Malayan falciparum infections is illustrated in the plate The form of the bodies accords fairly closely with Garnham s description they differ in that they are not always single (16, 21, 23), while the term ' thick bar seems hardly appropriate for the tubular appearance they present They occur at all stages in the early development of the gametocyte in association with the circular oat shaped or spindle forms but they are not constant, appearing only in a proportion of the gametocyte infected cells

Unusual appearances—Garnham bodies in cells doubly infected with circular or oat shaped gametocytes are shown in figures 31 33 Red cell inclusions with the appearances of Garnham bodies, occurring in cells which were not infected at the time are drawn as figures 35 and 36, there were many young gametocytes with typical Garnham bodies in the same film

Association with heavy infection—Garnham bodies are found in association with developing falciparum gametocytes and for this reason they are seen in blood films only in severe infections The examples shown in the plate were drawn from blood films taken from seven patients of whom three died from the infection

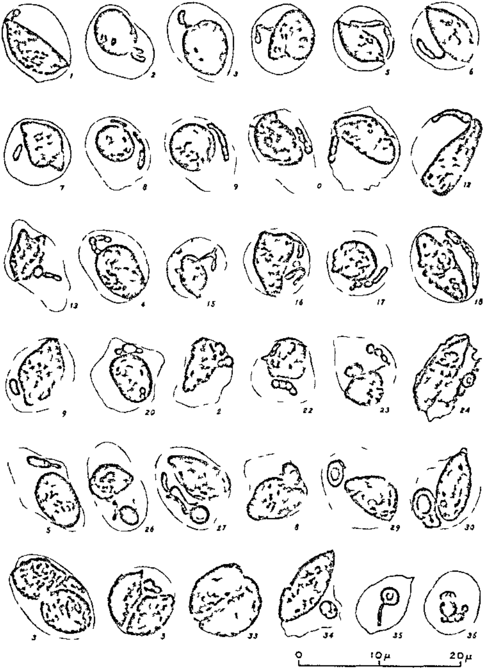
Origin—The nature of these bodies is unknown Garnham suggests that they may be derived from the parasite, representing some vestigial supporting structure no longer necessary to the organism Thomson and Robertson advance the theory that they are fragments of the capsule or periplast torn from the young gametocyte Thayer (1900) has portrayed the appearance of bud like processes which slowly protrude from the young live gametocyt and then retract, and it is conceivable that these processes may be detached from the main body of the organism during fixation Yet another theory is that they are derived from the host cell that they represent areas of detachment from the red cell surface of the growing and spreading organism—that they are in other words, the homologues in the gametocyte-infected cell of Maurer s spots in the cell infected with the schizogonic forms

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GARNHAM 1933 (ref p 140) Thomson and Robertson 1935 (ref p 140) Thayer 1900 (ref p 14)

THOMSON J G (1933) Peculiar bodies in red blood corpuscles containing developing crescents. *T ans R Soc trop M d Hyg* 27 7

XIV *Plasmodium falciparum*



GARNHAM BODIES IN THE HOST CELLS OF THE EARLY GAMETOCYTES

XV *Plasmodium falciparum* ADVANCED AND MATURE GAMETOCYTES

The full grown gametocytes of *Plasmodium falciparum* are the familiar crescents They appear in the peripheral blood late in the falciparum attack usually about ten days after the start of fever, but they are comparatively resistant to drugs and may afford evidence of malaria infection for days or weeks after the subsidence of symptoms and the disappearance of the schizogonic forms from the peripheral blood

Form—Laveran referred to the gametocytes of *P falciparum* as crescentic bodies, and the term crescent has since been generally accepted Yet the name is not a happy choice, the adult gametocytes are seldom crescentic, they are sausage shaped with rounded ends Plate XV illustrates a range of typical appearances in the males (1 21) and females (22 42) The gametocytes vary in length from 8μ to 12μ or more and in width from 3μ to 6μ, the females as a rule are longer more slender, and more gracefully curved, than the males Marked variations in form and size are common, some are adventitious produced in spreading the films some may express a natural variation Short, stumpy gametocytes male or female, are common in films which have dried slowly Forms of *P falciparum* in West Africa and Ethiopia which constantly produce small gametocytes have been regarded as special varieties (p 47)

Cytoplasm—The cytoplasm is the main substance of the parasite It varies in density often with a peripheral condensation which has been regarded as a periplast or capsule *In the females the cytoplasm stains blue and in the males a paler reddish purple*, but with routine methods of staining this colour distinction is not always clear [One of us (J W F) often fails to discern a difference which to Y L F is obvious]

Chromatin—In the female gametocyte the nuclear chromatin is concentrated as a purplish mass usually in the centre and often rather obscured by the pigment, in the male it is looser in texture and more widely diffused throughout the cytoplasm To this difference in the distribution of the chromatin is related the colour distinction between the stained males and females *

Pigment—The pigment is coarse It has the form of irregular granules or short rodlets, typically distributed around the centre of the parasite while the poles are pigment free As a rule the pigment in the male is more widely scattered than that of the female, a useful but not altogether reliable distinction of sex (plate XXXI) † Marked changes in the form of the pigment are common during treatment with mepacrine or chloroquine, the granules may coalesce into one or two irregular masses, and a complete absence of pigment is not unusual These changes are not shown in the plate (*see* p 240)

Host cell—The host cell of the mature gametocyte is usually a mere remnant It appears typically as the well known Laveran bib arched across the concavity of the parasite (1 7) Sometimes the bib is puckered (3, 4 6 7) and it may be absent (19) The host cell may also project beyond the convex border of the gametocyte as a false capsule either smooth (32, 40) or puckered (5 8, 24, 25) Bizarre appearances from a folding or distortion of the remnants of the host cell are common (12, 32, 36 41)

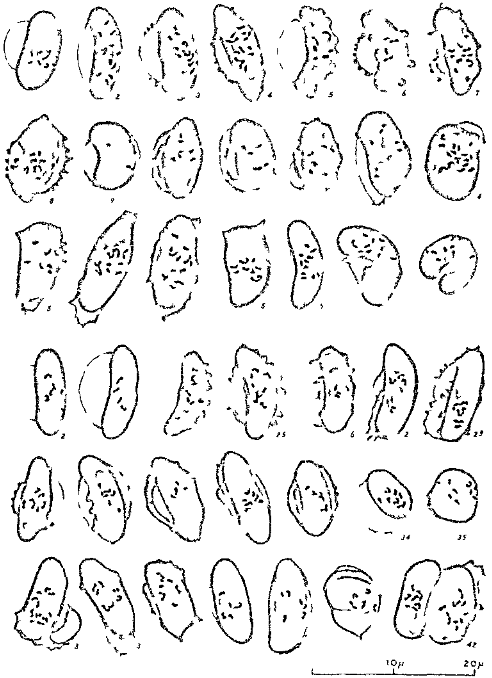
Double infections—Two full grown gametocytes, *female and male in close association*, apparently from invasion of the same cell, are shown in figure 42

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* Th nuclear chr matin in th gametocytes of Eur pean strains of *P falciparum* is more clearly d fined than that hown in th plat (P G S)

† Th ex di ti ction n th dispos ti f the pigment seem t be more mark d n the European parasites In the females the pigme t usually form a b ok n circle around the nucleus wherea n the male t is scattered loosely over about two-thirds of the parasi leaving only th poles free. These diff ences are regarded as reliabl indication of sex in the gametocytes of th European rgan m (P G S)

XV *Plasmodium falciparum*



ADVANCED AND MATURE GAMETOCYTES

XVI *Plasmodium falciparum* MATURATION CHANGES IN THE GAMETOCYTES

Profound changes in the gametocytes occur when they enter the stomach of the mosquito They pass through a ripening phase, a nuclear reorganisation in preparation for sexual union or syngamy The process is thought to be associated with a reduction division of the nucleus whereby the chromosomes are halved in number, the redundant nuclear material being discarded The complex and intricate mechanism of the presumed reduction division is not revealed by ordinary methods of examination, nor, indeed, has the process been clearly defined, but there are gross morphological changes during maturation which are easily seen in Romanowsky stained films Some of these changes are illustrated in plate XVI The drawings were made from thin blood films which had been kept in a moist chamber for several minutes and then fixed and stained by a modified Leishman method

Females (1-12)—An early change in the appearance of the female gametocyte is that which Thomson and Woodcock (1922) interpret as the escape and rounding up of the gamete The female gametocyte appears to have a fairly tough capsule, a bulge appears in the concavity (1) and the gamete escapes through a hernia in the capsule (4-7, 11) leaving behind the shell of the gametocyte which, in the examples shown, still contains some of the pigment Bizarre appearances from distortions or fragmentations of the cytoplasm are common

Clusters of small round bodies, pale pink in colour and apparently extruded from the parasite, are sometimes seen attached to the side of the gametocyte opposite to the escaping gamete They are well shown in figures 9 10 Thomson (1914) illustrates somewhat similar appearances

Males (13 30)—The apparent herniation of the gamete observed in the female seems to be absent in the male The capsule may bulge (13-15) but appears to yield evenly all round as the gametocyte becomes circular At this stage, the gametocyte stains a deep purple and the colour distinction between male and female is much more obvious than in ordinary blood films The curious extrusions, already noted in the females, are sometimes seen at the periphery of the males (16-21) the nature of this extruded material is not known to us

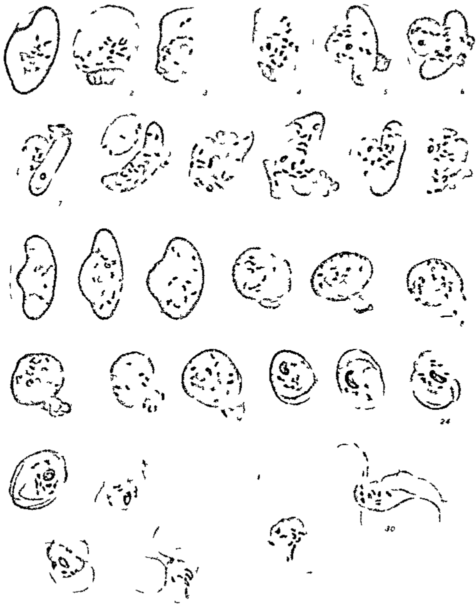
Polar bodies—After the reduction division of the nucleus, some part of the nuclear material becomes redundant Thomson (1914), and Thomson and Woodcock (1922) suggest that the redundant chromatin is extruded from the organism as *polar bodies* (plate II, 16-21) Casini (1936), on the other hand, believes that the superfluous chromatin of the male remains within the parasite, while the active generative chromatin moves to the periphery before dividing to enter the microgametes, only in the female is the redundant chromatin extruded The small deeply stained masses of extruded chromatin which Thomson and Woodcock describe as polar bodies have not been obvious in our material

Exflagellating males—The appearance of developing gametes tightly coiled around the male gametocyte like a dense thick capsule seems to be the first gross evidence of exflagellation in films kept in a moist chamber (22 23) Thereafter the gametes uncoil (24 27), until they are relatively free (28 30) The final detachment from the parent cell is not shown in the plate During this stage of gamete formation a well defined mass of chromatin is often strikingly obvious (22 -7) This mass remains when gamete formation is advanced and presumably represents the redundant nuclear material

Some of the gametes have small blobs of chromatin either along their course or as a terminal bead, others have no visible nuclear material Gametes without chromatin would be sterile, the nuclear material of some of these attached gametes is probably hidden within the parent gametocyte (*see* also plate XLVIII)

CASINI G (1936) Sul cos detto fenomeno dell l minazion dei globuli polari nei gametociti d l parassiti malarigeni *R v Mal n l 15 S z l* 88
THOMSON AND WOODCOCK 1922 (ref p 18) Thom n 1914 (ref p 138)

XVI *Plasmodium falciparum*



μ 20μ

MATURATION CHANGES IN THE GAMETOCYTES

Cerebral capillaries which appear to be blocked with parasitised red cells are often found at autopsy in subjects who have died from falciparum malaria, particularly when there were cerebral symptoms during life The parasites are usually presegmenters, segmenters, o merozoites, but they may be at any stage of growth The host cells tend to be packed tight together, with their individual outlines lost and in stained smears the parasites lie contrasted against a pale pink, more or less homogeneous, background of packed cells Plate XVII portrays the appearances of the parasites in different cases, figures 1 5 were drawn from smears figures 6-8 from sections

Figure 1 (*Case 1*)—The parasites are mature segmenters or scattered merozoites One segmenter lies outside the capillaries The pigment of each schizont is a single rounded mass, or a tight cluster of granules The dark ovoid or pyriform bodies are the nuclei of the capillary endothelial cells

Figure 2 (*Case 2*)—The parasites are larger than merozoites, they have grown to a stage at which in blood films they would normally appear as rings The compact appearance with slight vacuole formation is probably a post mortem change

Figure 3 (*Case 3*)—The patient was an elderly Chinese who died in coma soon after admission to hospital Two hours before death the blood was flooded with small rings The parasite density was 511 000 per c mm blood and 16 per cent of the red cells were infected. The brain smear was taken 16 hours after death The parasites are older than those portrayed in figure 2, in blood films they would appear as large ring form trophozoites

Figure 4 (*Case 4*)—The patient was a Chinese woman who was admitted to hospital in deep coma and died a few hours later The parasite density in the peripheral blood was 446 000 per c.mm 42 per cent of the red cells were infected The parasites are presegmenters and early segmenters about 36 hours old with well formed masses of pigment. These are the pigment masses which, ranged in lines along the capillaries, are so obvious in unstained material, even under the low power of the microscope

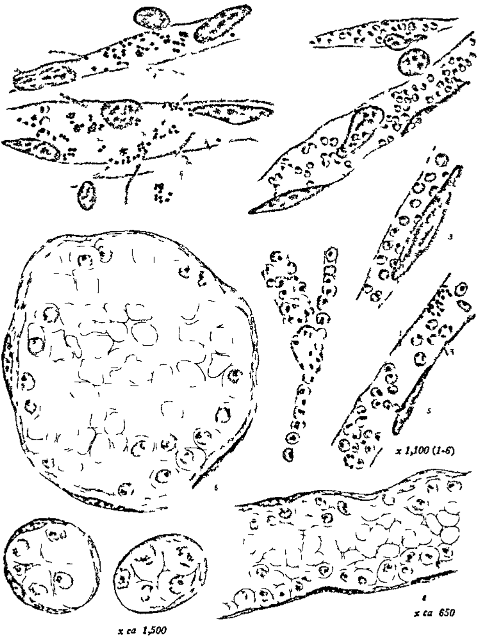
Figure 5 (*Case 5*)—Parasites of three broods were present in the brain smear from which this capillary was drawn. The dominant parasites were presegmenters, two parasites in early schizogony, and a cluster of merozoites may also be seen

Figures 6 8 (*Case 6*)—These drawings were made from stained sections They illustrate the concentration of parasitised cells around the periphery capillary walls, a phenomenon, illustrated very clearly by Mannaberg (1899), which may have an important bearing on the slowing down and final arrest of the blood flow

The growth phase of the parasites and the onset of coma—*Concentration of parasites* in the brain capillaries with an arrest of the capillary circulation might occur at either of two points in the blood cycle of the parasite—at the late trophozoite stage when the parasitised cells are caught up in the internal capillaries (4 5), or at the time of segmentation (1) The events in Case 3 (fig 3) suggest that segmentation was the time of particular danger Marchiafava and Bignami refer to a clouding of the sensorium with the segmentation of the organism Woino-Iassenetsky (1939), on the other hand states that coma begins 20-24 hours after the appearance of the generation of parasites responsible for the condition that is to say at about the time when the parasitised cells collect into clusters or adhere to the capillary walls From the limited material at our disposal we have not been able to correlate the onset of cerebral symptoms with any particular developmental phase of the organisms

MANNABERG J Die Malaria Krankheiten. A H lde W en, 1899
MARCHIAFAVA AND BIGNAMI 894 (ref p 3)
WOINO-IASSENETSKY M V (1939 La distribu ion des pa as tes d n l org nisme d l'homme au cours de la malaria tropicale M d Pa ant and P a i ic Di Moscow 8 3 (Abstract, T p Dis. Bull 1940, 3 861)

XVII *Plasmodium falciparum*



PARASITES AT VARIOUS STAGES OF DEVELOPMENT IN THE MINUTE VESSELS OF THE BRAIN

XVIII *Plasmodium falciparum* PHAGOCYTOSIS

Phagocytosis of the parasites in malaria infections is a striking phenomenon The large mononuclear leucocytes the wandering and fixed macrophages, and the polymorphonuclear leucocytes, engulf the parasites in vast numbers particularly at the time of segmentation The appearances have been interpreted to mean (*a*) that the phagocytes destroy living parasites and constitute an important, perhaps the most important means of natural defence, or (*b*) that they cleanse the vascular system by the removal of dead or degenerate parasites and their debris The emphasis, we believe, is to be placed on the active rather than the passive role

Phagocytosis is most active in the spleen the Kupffer cells of the liver and the bone marrow, but to a varying extent it occurs throughout the lymphoid macrophage system Phagocytes laden with parasites or pigment are often seen in blood films, but here they present a mere episode in a drama which is played out mainly in the viscera In the peripheral circulation the main phagocytic cells are the large mononuclear leucocytes, though the polymorphs are actively phagocytic in heavy infections, the lymphocytes and eosinophils display no phagocytic activity Macfie (1914) records a fatal falciparum infection in which two-thirds of the polymorphs in blood films contained either parasites or pigment, and Thomson (1935) states that a single polymorph may engulf as many as eight schizonts Taliaferro and Mulligan (1937) give a detailed account of the phagocytic cells involved in defence Thomson (1933) portrays phagocytosis by the large mononuclears and polymorphs in an excellent colour plate

Plate XVIII illustrates some of the appearances of phagocytosis by the polymorphonuclear and large mononuclear leucocytes in falciparum infections of Malayan origin the cells were drawn from blood films and placental smears

Phagocytosis by the polymorphs—The polymorphs seem to have a special avidity for mature schizonts and merozoites, or they may take up the pigment only The polymorphs shown contain (1) two masses of pigment from mature segmenters, (2) mature schizont (3) scattered merozoites, (4) two advanced schizonts, and (5) two immature schizonts and scattered merozoites Evidence in blood films of phagocytosis by the polymorphs is unusual except in heavy infections (Shute has often seen exflagellating male gametocytes engulfed in the poly morphs in moist films and blood clot from the mosquito stomach but never in ordinary blood films)

Phagocytosis by the large mononuclears—Phagocytosis by the large mononuclear leucocytes is very common, particularly at the time of segmentation Phagocytes which contain pigment only are illustrated in figures 6-8 and 10, the pigment is mainly in the form of compact masses derived from mature segmenters Whether the pigment represents the remains of parasites which were engulfed alive and digested, or whether the phagocytes have taken up the pigment from the plasma as a mere scavenging activity, is not easily decided If we assume that each pigment mass represents a digested schizont the defensive role of these cells must be highly significant The cell shown as figure 10, drawn from a placental smear, contains at least 30 pigment masses, the maternal infection was very light

Phagocytosis of schizonts is shown in figures 9 11, 12 The macrophage drawn as figure 9 contains two schizonts, one half digested and lying in a vacuole A similar vacuole containing a schizont which is relatively undamaged is portrayed in the macrophage drawn as figure 11

Evidence of phagocytosis by the large mononuclears may persist in blood films for a week or more after the disappearance of the parasites from the circulation and may sometimes p ovide the only decisive indication of previous infection

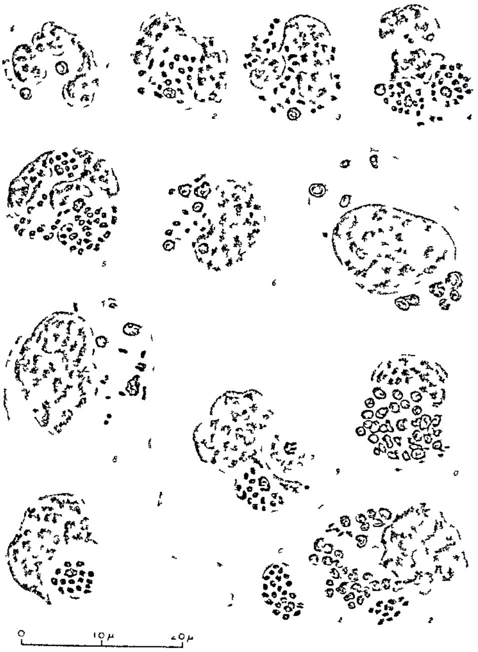
MACFIE, J W S (914) Note on a f tal and exceptionally inten i f ct with malaria (*P falciparum*) *Lance* 1354

THOMSON J G (1933) Immunity in malaria *T ns R Soc tr p Med Hyg* 6 483

THOMSON J G (1935) Demon tration of phagocytosis of th s hizonts f *Plasmod um f lciparum* by polymorphon clea cell *T ans R S tr p M d Hyg* 29 36

TALI FE RO W H AND MULLIGAN H W (937) Th his op thology of malaria with p cial r ference to the function and orig n of th macr phage in def n *Indian med Res M m* N 29

XVIII *Plasmodium falciparum*



PHAGOCYTOSIS

XIX DOUBTFUL SPECIES

Plasmodium tenue Stephens 1914

In a blood film from a child in the Central Provinces of India Stephens (1914) saw malaria parasites with young trophozoites which were highly amoeboid, with whimsical variations in form, the cytoplasm was scanty and the nuclear chromatin disproportionately large in amount These features, he thought, distinguished the parasite from any described before he regarded it as a new species and gave it the name *Plasmodium tenue*

Stephens was not the first to see these peculiar trophozoites They were portrayed in a colour plate by Balfour (1908) who regarded them as amoeboid forms of *P falciparum* while Welch (1897) in his description of *P falciparum* had written of the manifold shapes which the parasites may take in their amoeboid movements

The views of Stephens were supported by Sinton (1932) but criticised by others particularly by Mary Lawson (1916) Knowles (1923) and Callanan (1926) The grounds for criticism were (*a*) that tenue forms tend to be grouped in particular areas of films which elsewhere show normal falciparum rings (*b*) that films taken from the same patient at the same time may show tenue forms in some and ring forms in others, and (*c*) that tenue forms occur in all the malaria parasites infecting man In face of this criticism *P tenue* has not been accepted as a valid species and by most authorities tenue forms are now regarded as distortions in the trophozoites of *P falciparum* Whether the distortions are natural or artificial is not clear They may express an unusual amoeboid activity in the parasite, as Balfour and Wenyon (1914) believed, or they may be due to physical stresses imposed on the parasites when the blood is spread on a film the view so convincingly put forward by Mary Lawson (1916) and Callanan (1926)

Tenue forms of *P fal iparum* are fairly common in Malayan infections, usually accompanied in the same films by ordinary ring forms Like most distortions of the malaria parasites in thin blood films tenue forms are more common at the tail of the spread where the films dry quicker and the parasites have had further to travel A film from a Malayan falciparum infection in which many of the trophozoites had a typical tenue morphology contained more than thirty times more tenue forms at the tail end of the spread—70 per cent of the total trophozoites compared with 2 per cent at the butt Sometimes tenue forms appear in large numbers in a single film, one of us recalls an occasion when six films were taken from one patient at the same time, five films showed normal ring forms only while the sixth showed many tenue forms

The drawings in plate XIX, reproduced from original sources portray the common appearances of these tenue forms The cytoplasm is scattered in irregular wisps and strands and the nuclear chromatin is likely to show a similar if less marked distortion Sometimes the amount of chromatin appears to be excessive (19 21 25) The distortions are most marked in trophozoites of small or medium size, large trophozoites with ample cytoplasm in cells containing Maurer's spots, seem to be less liable to assume these extreme vagaries of form

STEPHENS 1914 (f p 51) B lfour 1908 (ref p 51)

BALFOUR AND WENYON 1914 (ef p 51)

SINTON J A (92) A ca e of malari d e to *Plasm d um tenue* (St ph n) *I di J m d R s 10* 215

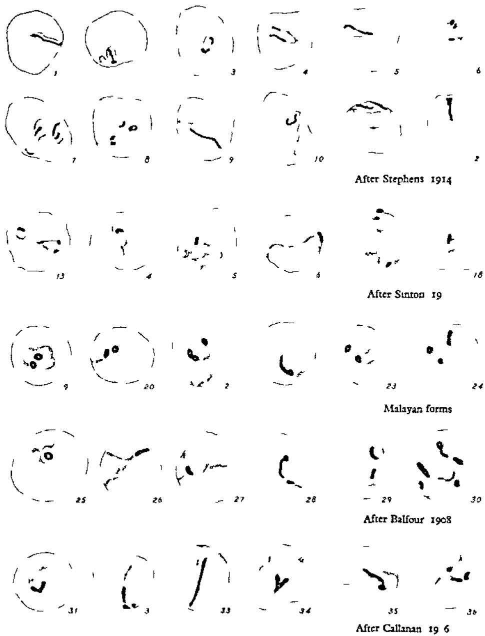
ROWLEY LAWSON MARY (1916) D t rtion f the malarial para te A nt rp etation of *Pl sm d um te ue* (Stephens) *J xp Med 4* 29

KNOWLES R (1923) Co pond e *I d a m d G 58* 500

CALLANAN J C J (1926) A s de-light n th p oduct f *Pla m d um t nu* (S ph) *Ken m d J 3* 18

THOM ON J G (9 8) D mo tr ti n f p epar tio showi g t f rm of malari par and Maur r' d ts in *Plasmod um fal p rum* inf ct *T ns R S trop M d Hyg 2 7*

XIX DOUBTFUL SPECIES— *Plasmodium tenue*



A COMPARISON OF *Plasmodium tenue* (1 18) WITH TENUE FORMS OF *Plasmodium fal iparum* (19 36)

XX DOUBTFUL SPECIES OR SUB SPECIES

The malignant quotidian parasite—First suggested by Grassi and Feletti (1890) the existence of a malignant malaria parasite with a 24 hour blood cycle was widely accepted at the turn of the century The parasite, it was thought, could be distinguished not only by the quotidian fever to which it gave rise but also by its morphology, particularly by the small size of the schizonts Marchiafava and Bignami (1894) portray the appearance of these schizonts (1 6)

Plasmodium falciparum quotidianum Craig 1909—This parasite found by Craig in the Philippines is said to resemble the malignant quotidian parasite of the early Italian workers The table below summarises the morphological difference from *P falciparum*

	P falciparum	*P falciparum quotidianum*
Earliest forms	Rings 1 5μ	Oval bodies 0 5μ
Ring forms	1 5μ 3 5μ Ample cytoplasm Small chromatin dots Fine pigment	0 5μ 1 0μ Scanty cytoplasm Ample irregular chromatin No pigment
Presegmenters	1 5μ 6 μ Pigment in granules or clumps	1μ 3μ Pigment in one or two solid blocks
Segmenters	5μ 6 5μ Pigment an irregular mass	3μ 3 5μ Pigment a solid block
Merozoites	Small dot of chromatin 10 13 in number	Mainly chromatin 6-18 in number

Figures 7 12 are drawn from Craig s photomicrographs The forms portrayed might well be considered to fall within the normal range of variation in *P falciparum* Early forms as small as 0 5μ the size mentioned by Craig we have never seen in the Malayan parasites

Plasmodium perniciosum Ziemann 1915—First seen in the Cameroons by Ziemann in 1896 this parasite is said to differ from *P falciparum* in having (*a*) a variable blood cycle of 24 48 hours, (*b*) lesser finer pigment which does not fuse into large masses in the schizonts (*c*) smaller segmenters with 6 12 merozoites, and (*d*) a lesser production of gametocytes The only illustrations of the parasite we have been able to trace are those redrawn as figures 13 15 from Mense s *Handbuch der Tropenkrankheiten* 1906 The differences from *P falciparum* are minor, and *P perniciosum* has not been generally accepted as a valid species

Plasmodium caucasicum Marzinowsky, 1916—This parasite was described by Marzinowsky (1916) from the blood of a child in the Caucasu The infection was heavy The forms illustrated in his paper, redrawn as figures 16-21, might well be normal or slightly variant forms of *P falciparum* with Maurer s spots unstained

Plasmodium vi ax var *minuta* Emin 1914—Described by Emin (1914) from the blood of Asian and African pilgrims living on the island of Camaran in the Red Sea as a small variety of *P vivax* this parasite was renamed *P camaranense* by Ziemann (1915) who thought that it resembled *P falciparum* more closely but differed enough to be regarded as a new species Some of Emin s drawings are redrawn as figures 22 23 On the evidence of the des criptions and drawings we see no good reason why the parasite despite its abnormal features should not be regarded as *P falciparum* the Schuffner like dots (26 27 30) we interpret as late Maurer s spots (*see* plate VIII) the circular gametocytes (32) as the intracorpuscular forms shown in plate XIII The infections were heavy schizonts and young gametocytes in the peripheral blood would not be unusual in these circumstances

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CRAIG C F (909) Cited by Craig (1926)
— (19 1) Th cla sificatı n and diff ntial diagnosis f th esti o- uumnal malaria pla modia. *Amer J trop M d* 1 57
ZIEMANN H *Mal ri n H ndbuch der T penkr khnten* I I 3 Ed C Men Johann Barth Le pzig 1906
ZIEMANN 19 5 (ref p 5) Marzinow ky 1916 (ef p 52) Emin 1914 (ref p 51)

XX DOUBTFUL SPECIES OR SUB SPECIES



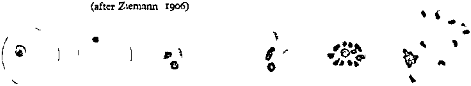
The malignant quotidian parasite
(after Marchiafava and Bignami 1844)



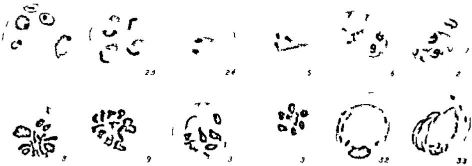
Plasmodium falciparum quotidianum
(after Craig 1921)



Plasmodium perniciosum
(after Ziemann 1906)



Plasmodium caucasicum
(after Marzinowsky 1916)



Plasmodium vivax var minuta
(after Emin 1914)

THE MALIGNANT QUOTIDIAN AND OTHER FALCIPARUM LIKE PARASITES OF UNCERTAIN IDENTITY

A blackwater fever variety —The view dies hard that blackwater fever may be due to some special kind of Plasmodium Wright (1920) finding piroplasma like forms, along with the ordinary ring forms of *P falciparum* in cases of blackwater fever from the Coorg Province of India, suggested that they might bear some special relation to the disease, indeed that they might represent a special blackwater producing 'variety' of the parasite (1 11) Other unusual appearances are shown in figures 12 18, including a linear form (12) anaplasma like forms with no cytoplasm (17), and two normal looking falciparum schizonts in the same cell (18)

Similar piroplasma like forms are shown by Thomson (1924) in a colour plate illustrating the morphology of the forms of *P falciparum* found in cases of blackwater fever observed in Rhodesia Thomson however, appears to regard these peculiar forms as incidental variations in normal *P falciparum*

The suggestion of Wright that these piroplasma like forms might have a special relation to blackwater fever has not been confirmed and the concept that blackwater fever is caused by a special variety of *P falciparum* seems to have been abandoned

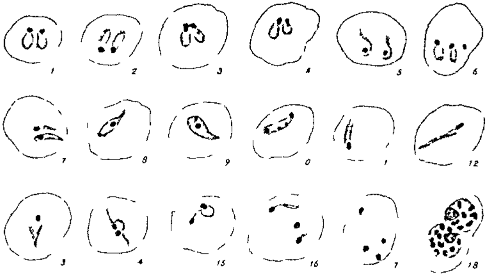
An Ethiopian variety—Between the forms of *P falciparum* found in Ethiopia and the typical Italian forms there are morphological differences which have led Raffaele and Lega (1937) to believe that the Ethiopian parasites may represent a special variety (see also p 50) The Ethiopian parasites are said to differ (*a*) in the larger rings, with coarser chromatin and a later appearance of pigment (31 33), and (*b*) in the shorter and broader crescents (males 25 27 females 28 30) The forms illustrated are common in *P falciparum* of Malayan origin (plate VI) (The authors refer also to biological differences, Italian anophelines were not easily infected with the Ethiopian parasites)

WRIGHT H (1920) A preliminary p p n b erv ti ns on bl ckwater fever (ha mogl binuria) in the Coorg Pr vince 9 7 1918 esp cially ; gards ts eti logical fact namely protozoan paras f th g nus Pir plasma in conjunction w th th malarial Plasmod um s a new and undescribed sp ci s f *Laver mo malarioa* (Don n theory) *Ind an m d Gaz* 65 161

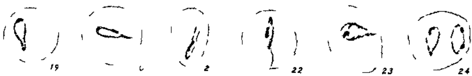
THOMSON J G (1924) R s arch n blackwa r fe e in S uthern Rhodesia Research M mo Vol VI *London S hool f T opical M d cin* pp 35 36

RAFFAELE G AND LEGA, G 1937) O rvazi ni su di un c ppo ti p o di P *f lciparum* *Ri M lari* 16 S z. 1 388

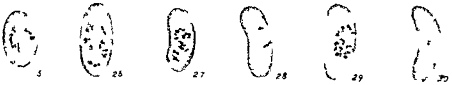
XXI VARIETIES OF *Plasmodium falciparum*



Piroplasma like and other abnormal forms in blackwater fever (after Wright 1920)



Piroplasma like forms in blackwater fever (after Thomson 19 4)





An Ethiopian variety (after Raffaele and Lega 1937)

AN ETHIOPIAN VARIETY AND A DOUBTFUL VARIETY ASSOCIATED WITH BLACKWATER FEVER

Invasion of host cells—The mature segmenters of *Plasmodium vivax* are seldom very numerous in the peripheral blood They tend to be held up according to Welch (1897) in the spleen and bone marrow We may thus infer that the first invasion of the erythrocytes does not always take place in the peripheral blood, and on these grounds we might perhaps explain the observation that the earliest forms seen in blood films often appear to be several hours old with the host cells slightly enlarged and early Schuffner's dots already visible

Form—The trophozoites of the Malayan parasite have the same morphology as those so fully described from other countries The youngest forms are small parasites 2 3μ in diameter They may have little or no vacuole (2), though typically the vacuole appears early, even in the merozoites (plate XXIV) and the young parasites then take the form of regular (3 5) or irregular rings (6 10) Two or more parasites may occupy the same cell (9 12) and very rarely up to five or more (plate XXVI) but multiple infections of the host cells are far less frequent than in *falciparum* infections Sometimes the trophozoites have a marginal attachment (13 14 16-17) especially according to Mackerras and Ercole (1949) when they are damaged during mepacrine therapy

Cytoplasm—In films which have dried slowly the parasites return to their resting form with the cytoplasm disposed as a fairly regular ring, but the young trophozoites are actively amoeboid and their changing form during life is often reflected in the varied cytoplasmic patterns of the fixed and stained organisms (23 36)

Chromatin—The typical form of the nuclear chromatin is that of a single circular or ovoid bead doubling or even trebling of the chromatin may occur but not very often (19-24) Marked distortions are unusual but linear and curvilinear formations are known

Pigment—Minute granules of pigment appear when the parasite is 6-12 hours old At first few and invisible as a rule in stained films they can usually be seen without difficulty at the later stages The granules are scattered throughout the cytoplasm often with a tendency to group around the vacuole or at the end of the pseudopodia (plate XXXII)

Enlargement of host cells—The host cells appear to enlarge within a few hours of invasion indeed the youngest parasites seen in the peripheral blood may be found in cells which are perceptibly bigger than normal A selective invasion of the reticulocytes may partly explain this early apparent enlargement but the marked enlargement which occurs later in the cycle is clearly related to the presence of the growing organism

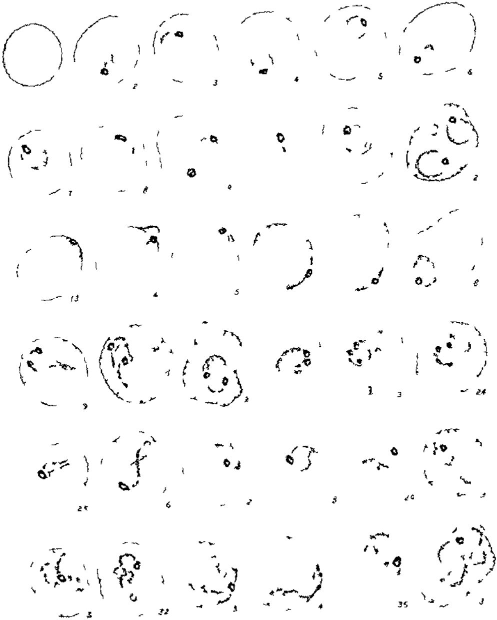
Colour of host cells—The pallor of vivax infected cells which is usual in fresh blood is seldom obvious in stained films Sometimes the infected cells have a purple tinge (plate XLIX) these purplish cells are probably reticulocytes

Schuffner's dots—The appearance of Schuffner's dots in Romanowsky stained films varies with the pH of the stain diluent (plate XXXVIII) Stained at a pH of 7 2 they can be demonstrated in nearly all cells infected with *P vivax* of Malayan origin they are absent only in proportion of the cells containing very young trophozoites We have reason to believe that many infected cells are already stippled on their first appearance in the peripheral blood The earliest dots are few and small (2 5) the dots increase in size and number and the infected cells are soon heavily stippled The dots are pinkish in colour fairly even in size and either discrete or vaguely trabecular They are better illustrated than described (plates XLIX LIV) Schuffner's dots are so constant and so distinctive in appearance that they are extremely useful in species diagnosis

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WELCH 1897 (*cf* p 46)
MACKERRAS M, J AND ERCOLE Q N (1949) Some observation on the action of quinine atebrin and plasmoquin on *Plasmodium vivax* *Trans R Soc trop Med Hyg* 42 443

XXII *Plasmodium vivax*



EARLY TROPHOZOITES

XXIII *Plasmodium vivax* ADVANCED TROPHOZOITES

The forms of *Plasmodium vivax* portrayed in the plate represent the intermediate stage in the schizogonic cycle of the organism In simple tertian infections trophozoites at this stage are likely to be found in blood films during the fever free interval between paroxysms

Form—During life, the parasites at the earlier stages shown in the plate are in incessant movement with continual changes in form These changes are reflected in the varied shapes of the fixed and stained organisms (1 15) The movements are more sluggish in the later stages, and the parasites are then more compact (16-30) The vacuole, relatively large at first tend to close at the late trophozoite stage but may persist until the approach of nuclear division

Cytoplasm—With the growth of the parasite, the cytoplasm increases in amount but remains varied in pattern especially at the earlier stages portrayed in the plate Large irregular rings (2 10) are usual, but marked irregularities in contour are very common (11 15) In the later stages the organism shows less evidence of amoeboid activity, and the advanced trophozoites usually have a rounded or ovoid form with a relatively regular outline and compact deeply stained cytoplasm

Nuclear chromatin—The typical nucleus of the 18 24 hour trophozoite, like that of the young trophozoite is a single rounded or ovoid bead (2 4 11 15), sometimes there are twin beads (6 7) With advancing growth the chromatin increases in amount and becomes looser in texture and more irregular in form, it lies as a rule near the periphery of the parasite and may be ovoid (21 27) or curved in varying degrees (16 28 30), or it may almost encircle the vacuole (19 20)*

Pigment—The granules of pigment increase in number and size as the parasite grows They are usually scattered throughout the cytoplasm with a slight tendency to collect around the vacuole and at the periphery of the organism (*see* plate XXXII) James (1934) has drawn attention to the coalescence of the pigment granules even the absence of pigment, during mepacrine therapy, these changes are not shown in the plate (*see* p 240)

Host cells—Erythrocytes containing the later trophozoites are almost invariably enlarged usually to a diameter of about 10μ they are typically rounded or ovoid but their contour is often irregular and they are readily indented by contact with the neighbouring cells In films which are fully stained at a pH of 7 2 they are always stippled with Schuffner s dots, sometimes so heavily as to obscure the finer details of the parasites

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* Th n l chromatin usually li in close association with th acu l a mutual rel tionship which recalls th [illegible] some of th e ly w k rs th t th hr matin is the n cl lu of an he m ti cul cleus A imilar pr ximity of cuole and nucl ar chr ma in is usual in *P f lcip rum* and *P m lariae* (pl tes VII and XXVIII)

JAMES S P (1934) The d ct ff ct of at b in on the paras t s of benign tertian nd quartan malaria *T n R Soc. trop Med Hyg* [illegible] 8 3

XXIII *Plasmodium vivax*



ADVANCED TROPHOZOITES

XXIV *Plasmodium vivax*—SCHIZONTS

Nuclear division in *Plasmodium vivax* begins when the parasite is about 42 hours old and is completed within the next six hours or so The whole process may be followed in films from the finger blood though the ripe segmenters are more numerous in the spleen than they *are in the blood from the finger* The young schizont is from one *half* to two thirds the size of the host cell (2-5) the mature schizont fills or nearly fills the cell (16-20) The schizonts are rather striking bodies They are compact and rounded or somewhat irregular in form with abundant cytoplasm and vividly stained masses of chromatin

Cytoplasm—With the change from the nutritive to the reproductive phase the parasites become less active they lose their vacuole, and tend to assume the rounded form of rest The blue staining cytoplasm at first rather dense, becomes looser in texture as the schizonts approach maturity (11 15) and finally condenses around the masses of chromatin to form the merozoites (16-20)

Chromatin—*The nuclear chromatin diffuse and ill defined in the presegmenters, is* strikingly obvious in the schizonts as dense masses which stain a deep reddish purple After the first division (2) the daughter nuclei tend to move to opposite sides (3), before the later divisions into from 10 to 20 or more segments The shape and size of the masses are variable usually rounded or ovoid (2 10) they may assume bizarre shapes related perhaps to mitotic changes (13)

Pigment—The granules of pigment in the young schizonts are usually scattered They begin to concentrate in the later stages of schizogony, and in the mature segmenters they cluster near the centre or periphery of the parasite (17 20) The granules are typically discrete a few may fuse together, but there is seldom the general coalescence to a singl solid mass which is usual in *P falciparum* (*see* also plate XXXII)

Merozoites—The merozoites are round or ovoid bodies, with a central or lateral mass of chromatin closely invested with cytoplasm Visual evidence of the beginnings of a vacuole is not unusual The merozoites drawn as figures 21 24 show a considerable variation in size, they *were drawn to the same scale from blood films taken from four patients three* infected with Malayan parasites and one with a parasite of Madagascan origin (24) We have no reason to believe that the differences go beyond the normal range of variation The number of merozoites in mature segmenters varies from ten or less to twenty or more with an average of about sixteen

	Aver ge	Rang
Malayan strain (Field and Niven 1939)	16	12 20+
New Guinea Chesson strain (Jeffery *et al* 1955)	17	10 24
American St Elizabeth strain ( )	14	9 20

Host cells—The host cells are markedly enlarged and heavily stippled with Schuffner's dots so heavily that the finer details of the structure of the parasite may be obscured Some irregularity in the outline of the cell is not uncommon (6, 11, 17 19)

Two types of schizogony—Gametocytes are usually regarded as offshoots of the schizogonic cycle Boyd (1935) and Corradetti (1936) recognise vivax schizonts of two types, the first type gives rise to the successive asexual generations the second may possibly produce merozoites destined to become gametocytes

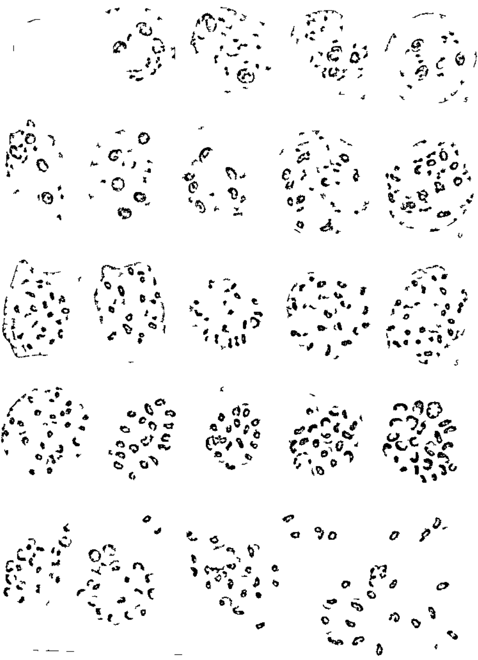
JEFFERY G M WILCOX AIMEE AND YOUNG M D (955) A comparison f W st Africa and West P cifi train f *Plasmod m ov l T ns R S tr p M d Hyg 49* 168

FIELD J W AND NIVEN J C (939) An malies in th m rph l gy f *P vi ax Inst med L 74* 309

BOYD M F (1935) On the schizogon us cycle f *Plasmod um ax* G and Fel tu *Amer J trop M d 15* 605

CORRADETT A (936) O rv zi ni ul cicl chiz goni o d l *Plasm dium viv x R v M lari I 15* Sez I 4

XXIV *Plasmodium vivax*



SCHIZONTS

XXV *Plasmodium vivax*—GAMETOCYTES

The gametocytes of *Plasmodium vivax*, developing mainly in the spleen are usually full grown when they enter the peripheral blood In primary attacks they *first appear in blood* films after several days of fever, but in relapses they may sometimes be found on the first day They are compact rounded bodies, with undivided chromatin and abundant scattered pigment The males may be distinguished from the females by their smaller size their paler, pinker staining, their larger and more diffuse mass of chromatin, and sometimes by differences in the distribution of the pigment

Young forms (2 5)—Though the early gametocytes of *P vivax* do not regularly appear in the peripheral blood they are not uncommon in films taken on the first day of relapses They are rounded, compact deeply staining bodies, with no vacuole The chromatin is a single mass of varied form, sometimes central or eccentric, often peripheral The pigment is scattered in fine granules At this early stage the sex is not easily determined

Adult females (6 10)—The full grown female gametocytes, or macrogametocytes, are large rounded parasites which fill or nearly fill the host cell The cytoplasm in stained films is blue and fairly homogeneous The nuclear chromatin is a single well defined, purplish mass varied in form and usually peripheral in distribution The nucleus is said to consist of chromatin granules set in a lightly stained nuclear sap which sometimes appears as a clear zone around the periphery The pigment is abundant and usually dispersed as small granules throughout the cytoplasm (plate XXXII)

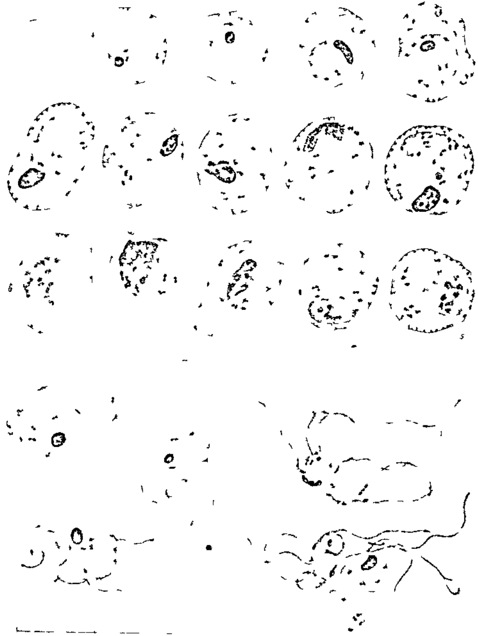
Adult males (11 15)—The adult male gametocytes the microgametocytes, are similarly recognsied as gametocytes by their compact and rounded form, by the absence of chromatin division and by the abundant scattered pigment (11 15) They may be distinguished from the females by the large loose, and ill defined mass of chromatin and by their paler colour and smaller size The pigment is scattered, with a special tendency to collect around the nucleus when examined by polarised light it may appear as a brilliant collar of granules around the nuclear chromatin—a characteristic feature according to Marchoux (1926) by which the males may be identified (plate XXXII) An unstained area sometimes surrounds the nucleus like a halo (15)

Exflagellating males (16 20)—Maturation changes may be observed in films which have been kept moist The drawings illustrate some of the changes in the exflagellating males The cytoplasm stains more deeply (16 17) and a single condensed deeply stained mass of chromatin may usually be recognised (16 18) The first suggestion of gamete formation appears in figure 16 with the gametes wrapped tightly around the periphery of the parasite they are partly free in figure 17 and in figures 18 20 they are fully formed though still attached to the body of the parent The appearances in figures 19 and 20 suggest a possible protrusion and escape of the gametocyte from a capsule they may equally well be interpreted as a mere deformation and fragmentation of the cytoplasm

Host cells—The host cells are enlarged, markedly so in the later stages of gametogony They are always heavily stippled with Schuffner s dots unlike the host cells of falciparum gametocytes which are never stippled The rim of the intact host cell can usually be seen around the full grown gametocytes and even with commencing maturation the stippled remains of the host cell may still be evident (16-19)

MARCHOUX E *Nouvea T te d M d cine e d The apeutiqu* Ed. A. G lbert t P Ca not. V *Palud sm* J B Baılhè e t Fil P s 926

XXV *Plasmodium vivax*



GAMETOCYTES

XXVI *Plasmodium vivax*—UNUSUAL OR BIZARRE FORMS

Aberrations in the form of *Plasmodium vivax* are fairly common They have usually been found in a single film, or on several occasions in the course of a single attack None of the aberrations illustrated in plate XXVI were shown to be constant in a succession of hosts they should probably be regarded as incidental and fleeting variations

Multiple infection of the host cells—The invasion of a single cell by several parasites is not common in vivax infections but extreme examples of multiple infection have been recorded Chalmers and Archibald (1920) found up to six rings in a single cell (6 11) Rodenhuis (1921), Das Gupta (1939) Field (1942) and Young and Eyles (1949) have recorded a similar aberration The explanation of the phenomenon is obscure The possibilities include (*a*) a selective invasion of reticulocytes, (*b*) a restricted choice of host cells from the localisation and stagnation of segmenters in particular capillaries, (*c*) the invasion of erythrocytes by clusters of incompletely separated merozoites (*d*) binary fission and (*e*) the random chances of multiple invasion in heavy infections

Displaced parasites—Parasites which are partly or even completely displaced from the host cells are sometimes seen in blood films The examples shown (18 22) are taken from Mary Lawson (1914 1914a) who regarded the occurrence as evidence of migration to fresh host cells Another explanation perhaps a more likely one, is that the parasites are displaced by the physical stresses which are inevitable when the blood is spread on a film

Abnormal gametocytes—Thomson and Woodcock (1922) describe certain peculiar forms of *P vivax* found in old standing relapsing infections (23 26) They have a large deeply staining nucleus, and they bear a general resemblance to male gametocytes We have seen somewhat similar appearances in normal male gametocytes when the films have dried slowly enough to encourage the early changes of maturation

Azurophilic rings in vivax infected cells—Loop or ring formations which are quite distinct from Schuffner s dots and have no obvious relationship to the parasites, are sometimes seen in vivax infected cells The appearances are shown in figures 27 29 two of the cells contain young vivax rings (27 28) the third portrays these curious rings in association with Schuffner s dots in a cell from which the parasite has disappeared (29) Similar rings have been found by Craik (1928) in films stained with Manson s stain The origin of these rings is unknown

Band forms—Parasites which stretch as a linear band across the host cells occur in all forms of malaria though they are common only in quartan infections The examples shown in the plate were drawn from Malayan material (30 31) Similar forms of *P vivax* have been described by Mary Lawson (1916) Samarasinghe *et al* (1944) and others Band forms may occur at random in any vivax infection and at any stage of growth they are uncommon and have no diagnostic significance

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CHALMERS A J AND ARCH LD R G (1920) Th t nu phs of *Plasm d um* a (G as 1 and F l tu 189) *J trop M d Hyg* 3 33

FIELD J W (1942) M rph l g cal vari tion in *Plasmod um v* x G assi and Fel tti 1890 *P asit lo y* 34 82

RODENHUIS Y (19) Dubbel en p rinf cti b j malaria t rtiana *Gen e k T jdschr v N derl Indi* 61 123 (*Ab t t T p Dis B ll* 192 18 34)

DAS GUPTA B M (1939) Some anomali in the morphol gy of *Plasm d um v* occurring n a n wborn b by *Ind m d G* 74 273

YOUN M D AND EYLES D E (1949) Parasites es mbling *Plasm d um ov l* in strain of *Plasm d um vi ax* *J na M l r S* 8 9

LAWSON MARY R *J xp M d* (9 4) 19 45 (914a) 19 523 (1916) 4 291

THOMSON J D AND WOODCOCK H M Th p t f m lari Chapt 69 n *Th P ice f M d ci in th T p s* Ed W By m and R G Arch bald Henry F owde and Hodd and S o ght Lo don 9 2

CRAIK R (1928) Demo tr ti by D P H Man Bahr of ur phili ings infect d cell *T R Soc t p M d Hyg* 6

SAMARASINGHE D S PLVASEN A K AND RI STOMJEE K J (944) *P t nu f rm* and band f ms f *P vi x ee in* Ceylon du ing th p d m c f 1943 *J M l Ins Ind* 5 397

XXVI *Plasmodium vivax*



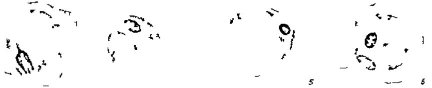
After Chalmers and Archibald 19 0



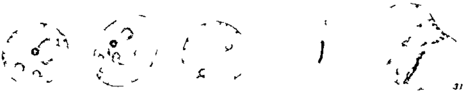
After Field 194-



After Mary Lawson 1914 1914a



After Thomson and Woodcock 19-2



UNUSUAL OR BIZARRE FORMS

XXVII *Plasmodium vivax*—UNUSUAL OR BIZARRE FORMS

Forms of *Plasmodium vivax* resembling *P ovale* are not uncommon Prolonged asexual propagation in *P vivax* either in chronic infections or from repeated blood passage of the organism congenital infection suppressive drugs, a high parasite density, are all possible causes of occasional ovale like characteristics The resemblance may be close but the changes are impermanent, and a reversion to a normal vivax morphology is likely when the organisms are restored to their usual environment and way of life

A small form of *P vivax*?—The forms reproduced as figures 3 6 were found by Macfie and Ingram (1917) in blood films from a child in the Gold Coast The authors identify the parasites as *P vivax* var *minuta* described by Emin (1914), but their resemblance to *P ovale* is very close Figure 2 portrays a stippled cell which has lost its parasite, a fairly common occurrence

Ovale-like morphology from repeated blood passage—Abnormal forms resembling *P ovale* have been found in vivax strains repeatedly passed by blood inoculation to fresh hosts with a prolonged exclusion of the sporogonic cycle The Wagner Jauregg strain isolated in 1919 in the Balkans and passed for fifteen years by inter human transfer, produced fewer merozoites the trophozoites were more compact and there was a lesser enlargement of the host cells (Giovannola, 1935) The drawings reproduced as figures 7 12 are taken from Missiroli and Siniscalchi (1929), they portray the schizogonic forms of a vivax strain from the Pontine Marshes which had been modified by six inter human transfers

Ovale-like schizonts in heavy infections—Abnormal schizonts with a reduced number of merozoites have been found in normal vivax infections at the time of maximum parasite density The ovale like forms portrayed as figures 13 17, were found by Young and Eyles (1954) in films from patients infected with the Chesson or Pait strains of *P vivax*, the abnormal schizonts (15 17) usually appeared at the time of maximum parasite density Shute and Maryon (1954) record the same phenomenon in heavy infections with a Madagascan strain

Ovale-like forms in congenital infection—Das Gupta (1939) describes the abnormal forms of *P vivax* found in films from an Indian infant fifteen hours after birth (20-22) Field (1942) records a similar aberration in the forms of *P vivax* found in blood films from an Indian infant sixteen days old (23 27) In both infants the infections were heavy and it is possible that the small number of merozoites in the schizonts was related to the high parasite density

Ovale like forms in chronic or suppressed infection—A tendency to morphological aberration in the parasites found in chronic vivax infections has long been recognised The schizonts may be of small size with but few merozoites and the host cells may show little enlargement (James, 1920, Giovannola 1935) A similar change is possible when the infections have been long suppressed by drugs Field (1941) describes an ovale like parasite of Malayan origin found in an Indian who had been taking suppressive mepacrine, figures 28 30 portray some of the abnormal forms

MACFIE J W S AND INGRAM A (1917) Ob rv ti on mal ri n th Gold Co Col y West Africa *Ann tr M d Paras t 11 1*

GIOVANNOLA A (1935) *Plasm d um l* cons dered modificati n f *P asm d um* ft a long esiden in th human host *Amer J tr p M d 15 75*

MISSIR I A AND SINISCALCHI R (19 9) Sull m dificazioni mo f l gi h b ogich d paras ti malarigeni n trapianti int rumani, *R M l ri l 8 25*

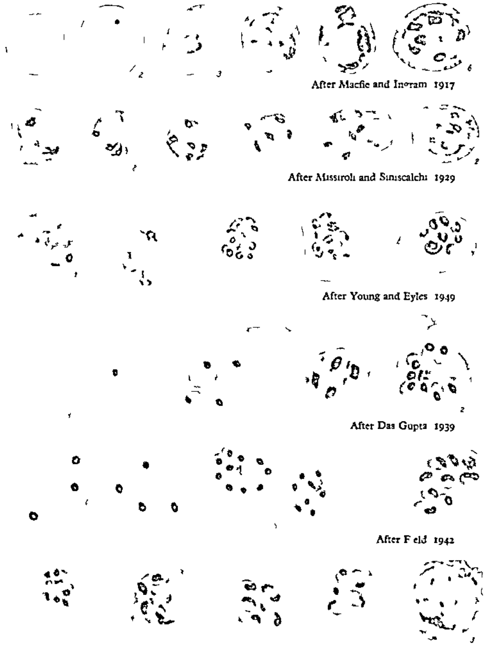
YOUNG AND EYLES 1949 (ef p 64) DAS GUPTA 1939 (ef p 68) FIELD 942 (ef p 68)

FIELD J W (941) A Mal yan p as t morph l gically esembling *Plasmod um ov l* S ph ns 19 2 *Bull Inst med R* Fed *Mal y S t* N 4 f 1941

SHUTE P G AND MARYON M. (1954) Ob erv tions on *P ov l* *Proc 5th C gr I ernat trop M d Mal* Istanbul 44

JAMES S P *Mal ri t Hom nd Ab d* J hn Bal S ns and Danielsson Ltd Lo don, 1920

XXVII *Plasmodium vivax*



UNUSUAL OR BIZARRE FORMS

XXVIII *Plasmodium malariae*—TROPHOZOITES

The quartan parasites in Malaya are similar in form to those described so *fully from other* countries, and little can be added to the masterly descriptions of the early Italian workers Typically they are compact, heavily pigmented parasites in cells which are unchanged in appearance They are less active during life than are the other species infecting man, and for this reason they are less liable to morphological irregularities

Rings—The earliest trophozoites in their typical form are fairly large regular rings with a single bead of chromatin (3 6) They are found in blood films during the paroxysm or a little before or after The chromatin is usually on the periphery of the ring (2 3) but the so-called bird s eye ' form with the chromatin in the centre of the vacuole is fairly common (5 6) The vacuole is often decolourised Multiple infection of cells is unusual but the presence of two rings in a cell with no other infected cells in the microscopic field, has been noted as an occasional and rather curious occurrence

Bands—Parasites which stretch across the host cells as equatorial bands are very common in quartan infections They occur at all stages of development from young and slender bands (10 11) to broad bands occupying most of the host cells (17 18) Sometimes they have a definite vacuole (12, 16) but more often the vacuole is flattened and invisible The nuclear chromatin is often stretched out as a line along the band The cause of this curious band *formation is not fully known the bands are not seen in lysed thick films, and it is probable* that they are artificial distortions of the organisms produced when the blood is spread as a thin film The bands usually lie oblique to the line of spread of the film, but they have no constant orientation, and they are dispersed with little tendency to occur in groups They are more common at the thinner tail of the spread where the parasites have dried quicker and travelled further Distortions, alike in the malaria parasites and in their host cells, are to be expected when the cells are rolled and pushed along a slide in the preparation of thin films The host cells from their elasticity quickly resume their normal shape and so do most of the parasites, particularly in the slower drying parts of the films The quartan organisms, we may presume *are less resilient than other species they recover more slowly and the distortions* tend to be fixed as the films dry

Rounded or ovoid forms—Amoeboid activity ceases when the live trophozoites are advanced in growth the organisms in stained films then appear as rounded or ovoid bodies of even and clearly defined outlines (28 36) The presence of a vacuole is usual (31 36) but not invariable (30) the vacuole tends to show a marked pallor and to persist to a late stage (34 36)

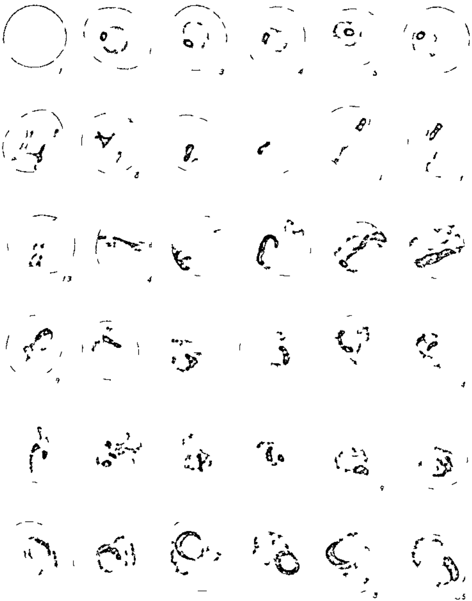
Irregular forms—Trophozoites which conform to no regular pattern are not uncommon They may have a tenue form (7 9) or they may be fairly compact parasites with angular irregularities (26 27)

Nuclear chromatin—The nuclear chromatin in the early ring forms is typically a single bead sited on the ring or quite often within the vacuole, but considerable irregularity is usual in the later stages The common patterns are illustrated in the plate

Pigment—The formation of pigment begins when the parasite is about twelve hours old and continues until the organism relative to its size is the most heavily pigmented of the species infecting man The granules are scattered throughout the cytoplasm sometimes with a tendency to collect around the periphery (32 36)

Host cells—The erythrocytes invaded by *P malariae* are usually unchanged in size shape and staining sometimes they appear to be smaller than normal (15 18) Deep staining at a pH of 7 2 may reveal the light stippling known as Ziemann s dots (6 8 28 30) but the dots are inconstant and of no diagnostic value

XXVIII *Plasmodium malariae*



TROPHOZOITES

XXIX *Plasmodium malariae*—SCHIZONTS AND GAMETOCYTES

Nuclear division in *Plasmodium malariae* begins when the parasites fill or nearly fill the host cells, some 48 60 hours after the beginning of the cycle The typical schizonts in stained films are compact, rounded, bluish bodies with vivid purple segments of chromatin The mature gametocytes are rounded heavily pigmented bodies of similar size but the nuclear chromatin is undivided Band forms of schizonts and gametocytes are not uncommon The host cells are unenlarged and usually unstippled

SCHIZONTS (2 24)

Cytoplasm—The vacuole often present in the late trophozoites disappears before the first nuclear division and the blue stained cytoplasm of the schizonts is fairly uniform until a late stage in the cycle Marked variations in morphology are unusual The parasites are usually rounded in form with a well defined outline Broad band forms, or a tendency to a band formation are fairly common (3, 4 7) The final condensation of the cytoplasm around the chromatin segments begins within the last few hours of the cycle

Nuclear chromatin—The nuclear chromatin, somewhat loose in texture in the presegmenters condenses with division into sharply defined segments which stain a vivid purple The segments are often irregular in shape and size particularly at certain phases of division (8 10) but with approaching maturity a regular ovoid or rounded form is usual (11 12)

Pigment—The schizonts are fairly heavily pigmented The granules are scattered throughout the cytoplasm with little tendency to concentrate until a late stage in the cycle In the mature segmenters the granules are usually collected into a cluster in the centre of the organism (20) though they may remain scattered over a considerable area (21) sometimes they fuse into a single dense mass (22 24)

Mature segmenters—The typical segmenter has eight merozoites arranged around a central collection of pigment granules—the well known rosette formation (20-21) But the arrangement is often irregular (22 24) and the number of merozoites may vary from six to twelve

GAMETOCYTES (25 36)

The early gametocytes of *Plasmodium malariae* are omitted from this plate They resemble advanced trophozoites and we have little confidence in our ability to identify them The adult male gametocytes (25 30) are round ovoid or sometimes band like bodies which fill or nearly fill the host cells The cytoplasm in stained films is rather pale and the chromatin is an ill defined pinkish mass placed as a rule to one side of the parasite The pigmentation is profuse with the granules scattered throughout the cytoplasm The female gametocytes (31 35) have a bluer cytoplasm than the males and the nuclear chromatin is smaller and more compact No distinctive differences in the appearance or disposition of the pigment in the males and females have been seen in the Malayan organism, nor have we noted the difference in size which is usual between the male and female gametocytes of *P vivax* The most reliable distinctions of sex are the differences in the colour of the stained cytoplasm and in the size texture and staining of the nuclear chromatin An unusual combination of a female gametocyte and a schizont in the same cell is portrayed in figure 36

XXIX *Plasmodium malariae*



SCHIZONTS AND GAMETOCYTES

XXX *Plasmodium ovale*

Stephens in 1922 described a parasite he named *Plasmodium ovale* from the oval shape of many of the infected cells The organism somewhat resembled *P malariae* but the host cells were stippled with Schuffner's dots The morphological characters were portrayed in a fine colour plate from which figures 2 20 have been redrawn

Young forms—The small rings are similar to the rings of other species or else regular and rounded forms with little vacuole The host cells are often oval, with a fringed margin

Half-grown forms—These forms resemble quartan parasites in their appearance of solidity, and in the amount of chromatin They do not assume the fantastic straggling forms which are so characteristic of *P vivax* at this stage, and they occur so often in oval red cells that this peculiarity can hardly be a matter of chance

Segmenting forms—The merozoites are at most twelve in number, and sometimes as few as six The host cells either normal in size or slightly enlarged

A more detailed description is that of James *et al* (1933) who recognised other features by which the parasite might be identified Figures 21 29 have been redrawn from the plate which illustrates their account

Young forms—The host cells are heavily stippled with Schuffner s dots at a very early stage The mass of chromatin is relatively large and of irregular shape

Half grown forms—The host cells are slightly enlarged and many are of peculiar shape with a margin which in parts may be drawn out into ragged points

Young schizonts—About a quarter of the host cells are oval in shape, and the general picture is that of a round parasite *in the centre of an oval cell Some host cells are* fringed with Schuffner s dots apparently extending beyond the margin

Segmenting forms—The general picture is that of eight merozoites arranged round a central block of pigment in a corpuscle heavily studded with Schuffner s dots

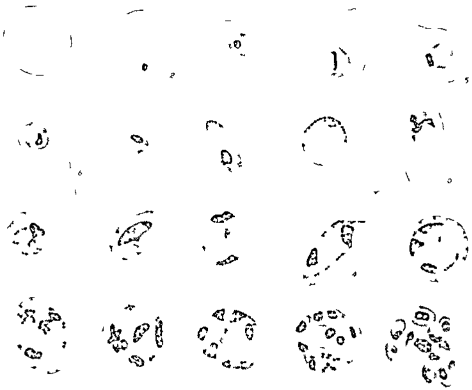
Gametocytes—Though not readily distinguished from vivax gametocytes, a visual comparison reveals differences in the colour and character of the pigment

Later accounts supplement these basic descriptions Wilcox *et al* (1954), and Jeffery *et al* (1955) have defined the differences in size between the West Pacific Donaldson strain of *P ovale* and the New Guinea Chesson strain of *P vivax* Garnham *et al* (1955) record their observations on an ovale strain from West Africa

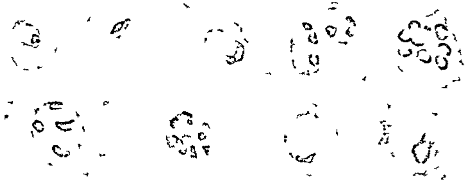
- *Segmenting forms*—The merozoites are larger than those of *any other species Some* schizonts found in relapses have 12 16 masses of chromatin and their host cells are grossly enlarged
- *Gametocytes*—When mature the gametocytes fill the host cells, the pigment has a distinctive arrangement of concentric rodlets particularly at the periphery
- *Host cells*—The oval shape and the fimbrication of the host cells though recognised as useful aids to the recognition of the species were thought to be artefacts occurring in dried fixed thin films

STEPHENS J W W (1922) A n w malar paras te f man *Ann p M d P an 16* 383
JAMES S P N COL W D AND SHUTE, P G (1933) *Pl m d um ov l* Stephe s 19 *P ant l gy 25* 87
JEFFERY *e l* 1955 (f p 64) WILCOX *e l* 1954 (f p 76) GARNHAM *et al* 955 (ef p 77)

XXX *Plasmodium ovale*



After Stephens 192



After James *et al.*, 1933

XXXI MALARIA PIGMENT—*Plasmodium falciparum*

When malaria parasites grow at the expense of cells containing haemoglobin they produce as a metabolic by product an iron containing pigment chemically related to haematin This pigment sometimes known as haemozoin first appears as minute granules in the cytoplasm of the ring forms The granules grow bigger as the parasite grows finally taking a form and arrangement which is characteristic of the species and phase The plate portrays the common patterns of the pigment in *Plasmodium falciparum* For greater clarity the chromatin and cytoplasm of the parasites are shown only in outline, and Maurer s spots are omitted

Ring forms—Pigment first appears as half a dozen or so minute granules in the ring forms which have grown to about the size shown in figures 1 6 The granules have a pale yellowish colour and soft outlines, and in stained films they can usually be seen only as a faint greenish haze in the blue cytoplasm

Late trophozoites—The granules are larger and have already begun to concentrate (7 12) They are now clearly visible even in stained films This early concentration of the pigment in the asexual blood forms is characteristic of *P falciparum*

Early schizonts—The granules have now run together to form a rounded mass with hard outlines (13 18) The colour has changed from the soft pale yellow of the early trophozoite stage to a dark yellow or mahogany brown

Mature schizonts—The pigment of the mature schizont is typically a single, rounded solid looking mass of unmistakable identity (19 24) The mass is usually central, but may be eccentric, or peripheral With bright illumination the mass appears to consist of a cluster of darkish granules in a lighter yellowish matrix This is the pigment which in severe falciparum infection is so often seen in the large mononuclear cells and even in the polymorphs Fresh unstained squash preparations of the brain from cases of cerebral malaria may show these pigment masses ranged along the lines of the capillaries, they are easily recognised from this characteristic distribution even with a low power objective

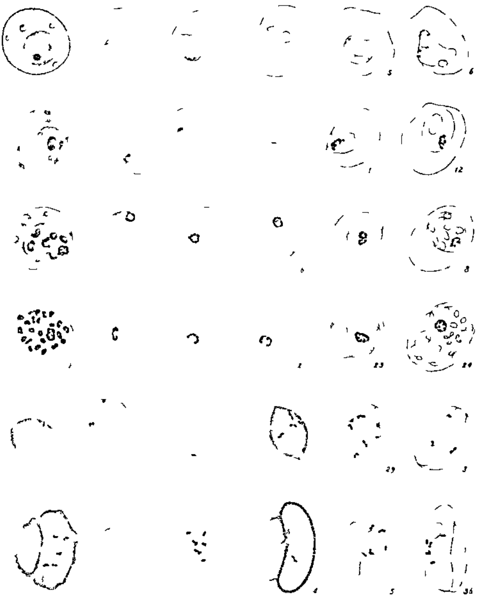
Young gametocytes—Watson (1903) working in Malaya recognised a very early stage of the gametocytes with one or two tiny granules of pigment (*plate XII*) *Plate XXXI* shows the pigment distribution at a later stage when the identity of the parasites is not in doubt The rounded intra corpuscular forms (25 27) and the oat shaped or spindle forms (28 30) are fairly heavily pigmented The granules about twenty in number are slightly elongated to a rodlet form and rather coarse they are scattered irregularly throughout the cytoplasm though the poles of the spindle forms may be pigment free

Mature male gametocytes—The granules from 12 24 in number, have a coarse rodlet form (31 33) They tend to collect towards the centre of the parasite leaving the poles free, though they are still scattered over a fairly wide area

Mature female gametocytes—No distinctive differences between male and female gametocytes, in the size shape, or number of the pigment granules have been observed But the concentration of the granules in the females tends to be greater and they tend to cover a lesser area (34 36) In the Malayan organism we have not found this difference to be a very reliable indication of sex Sometimes the granules are ranged around the mass of chromatin (35)

Abnormal or absent pigment—The pigment of the schizonts is normally condensed to a single mass at an early stage in nuclear division Jolly (1936) portrays schizonts in which the pigment granules are scattered to a late stage (plate XI) A malignant malaria parasite which produced no pigment during the schizogonic cycle was recognised by some of the early workers (*see* p 7)

WATSON M 903 (f p 14) JOLLY A M D 1936 (f p 28)



Plasmodium falciparum

XXXII MALARIA PIGMENT—*Plasmodium vivax*

The pigment of *Plasmodium vivax* appears in the cytoplasm of the ring forms at about the same stage in the cycle as that of *P falciparum*, but the appearances differ significantly as the parasites grow The *granules of vivax pigment are more numerous and more widely* scattered, they concentrate late in the cycle and seldom coalesce, and they remain scattered in the mature gametocytes The tracings reproduced in the plate were made from lightly stained parasites, the cytoplasm and chromatin are indicated in outline and Schuffner s dots are omitted

Trophozoites—The pigment first appears in the cytoplasm of the young trophozoites when they are 6 12 hours old (2) At first a soft pale yellow in colour and difficult to see in stained films, the granules increase in number and at the stages shown in figures 3 5 they are clearly visible as a greenish haze in the stained cytoplasm Throughout the trophozoite stage they have an uneven scatter, often leaving areas which are pigment free They are widely dispersed at a stage in the cycle when falciparum pigment has already concentrated to a tight cluster of granules (6 10) At this stage, and indeed at all stages the granules are not so clearly defined in stained films as they are in the drawings, they are veiled by the blue staining of the cytoplasm, and often obscured by Schuffner s dots *

Young schizonts—The form and distribution of the pigment show little further change The granules have about the same size and number and they are still dispersed with an uneven scatter among the chromatin segments (11 15)

Mature schizonts—The granules begin to collect into a loose group when the schizonts are approaching maturity (16 18) they concentrate at a late stage in the cycle (19) and in the fully ripe schizont they may fuse into a composite mass (20) but the tendency to coalescence is much less marked than it is in *P falciparum* at this stage, and even in the fully mature schizont the granules, though collected into a close cluster may still be discrete

Male gametocytes—Numerous granules of pigment are scattered throughout the *cytoplasm of the male gametocytes (21-25) Compared with the pigment of falciparum* gametocytes the granules are smaller and hence brighter in colour, and at no stage do they collect towards the centre Marchoux (1926) states that an arrangement of the granules like a collar around the nucleus is characteristic (24 25)

Female gametocytes—The female gametocytes (26 30) are profusely *pigmented The* granules are scattered widely and unevenly throughout the cytoplasm with no distinctive localisation No constant differences in the form of the granules between males and females have been observed Marchoux (1926) refers to the scattered needles' of pigment in the female gametocytes we have *not seen this appearance in the Malayan organism though some* of the granules may be rather elongated

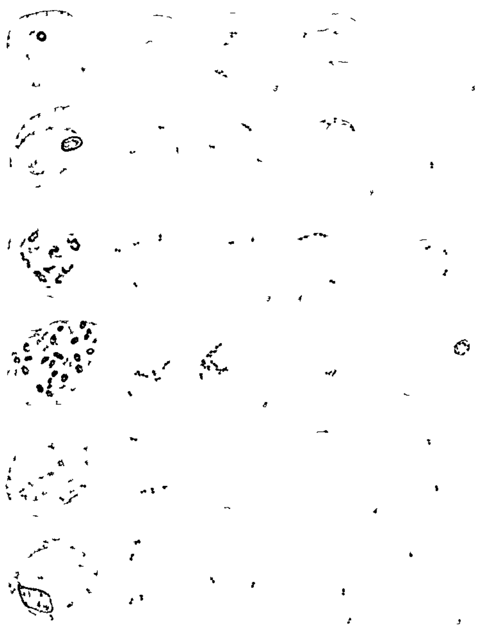
Abnormal pigment—A marked change in the appearance of the pigment may be associated with mepacrine or chloroquine therapy The granules tend to run together into clumps, or the parasites may lose their pigment altogether *(see* p 239) *Mohi ud din* and Rahim (1951) describe schizonts with no pigment the unpigmented schizonts were seen before treatment in a single case Yoeli (1948) records the occurrence of pigment free schizonts and gametocytes in the bone marrow

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* P gm t best seen n un tain d films or films tain d lightly that Schüffn dots ar n t vis bl and th cytoplasm ty pal *Bright llumin ti n mpha es th di tincti y ll wish colour Th pigm* t fl uresces und p larised light and detail d studi are aided by the use of Polar d filters (p 94)

MARCHOUX 1926 (cf. p 166) MOHI UD-DIN and RAHIM 1951 (cf p 64) YOELI 1948 (cf p 65)

XXXII MALARIA PIGMENT



Plasmodium vivax

XXXIII MALARIA PIGMENT—*Plasmodium malariae*

Relative to its size, *Plasmodium malariae* is the most heavily pigmented of the malaria parasites of man The amount of pigment is not more than that of *P vivax*, and it may be less, but it occupies a smaller area The granules resemble those of *P vivax* they are usually said to be coarser and darker, but this difference is not obvious in the Malayan organism The plate portrays some of the common patterns, other appearances are shown in plates XXVIII and XIX

Ring forms—The pigment first appears as a few pale yellowish granules with soft outlines in the cytoplasm of the ring forms In unstained or lightly stained films the pigment may be seen when the rings are about twelve hours old (2 3) The granules increase in number as the parasites grow and in the older rings they are visible in fully stained films as a greenish haze in the cytoplasm (4 6) The drawings were made from films stained very lightly in order to sharpen the definition of the granules

Advanced trophozoites—The granules of pigment are now fairly numerous and in relation to their size the parasites are heavily pigmented (7 12) The granules are dispersed throughout the cytoplasm sometimes with a tendency to collect around the periphery (8 10) With the illumination and optical system we employ the pigment appears to be greenish or yellowish depending on the depth of the cytoplasmic staining

Early schizonts—Descriptions of *P malariae* often refer to the withdrawal of the pigment to the centre of the parasite when nuclear division begins Little concentration of the granules occurs in the Malayan organism at the early stages of schizogony (13 18) the granules are usually dispersed, though they sometimes collect into loose groups (plate XXIX, figs 8 10, 14)

Advanced and mature schizonts—The pigment granules in the full grown schizonts may remain dispersed to a late stage (20), or they may collect into a loose group in the centre of the parasite (21, 22) A radial arrangement of the granules between the chromatin segments has been described this appearance occurs but is not usual in stained preparations of the Malayan parasite When the merozoites are fully separated, the pigment granules may be fused to a single rounded mass (24), sometimes coalescence of the pigment occurs a little earlier (23)

Male gametocytes—The male gametocytes are profusely pigmented The granules are numerous and, as a rule widely and unevenly dispersed (25 30) The area represented by the nuclear chromatin tends to be relatively free from pigment (26, 27, 29)

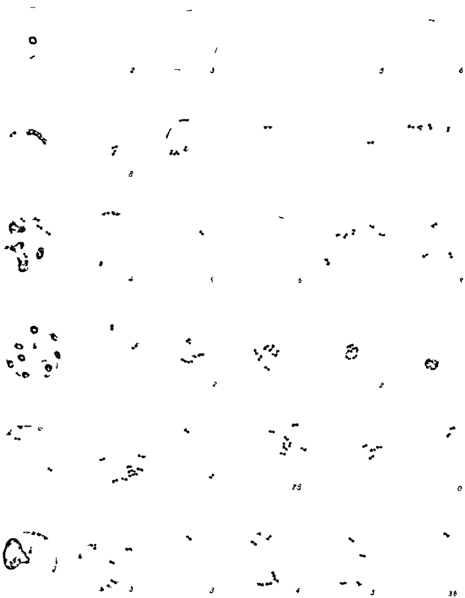
Female gametocytes—No consistent differences in the form, colour or arrangement of the pigment in male and female gametocytes has been observed in the Malayan organism (31 36) The granules sometimes tend to collect around the periphery, both in the femal and in the male gametocytes and even in unstained films the appearance of granules which are so disposed as to delineate the shape and size of the parasite is often distinctive enough to identify the species

Abnormal pigment—The form of the pigment, particularly in the advanced trophozoites and gametocytes is modified during treatment with mepacrine or chloroquine (*see* p 241) Garnham (1936) refers to differences from the usual appearances in the pigment of the quartan parasites found in a native reserve in Kenya, the granules were golden yellow rather than black

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GARNHAM 1936 (r f p 73)

XXXIII MALARIA PIGMENT



Plasmodium malariae

XXXIV PUZZLES AND FALLACIES IN BLOOD EXAMINATION—DEMILUNE CELLS AND PESSARY BODIES

Demilune cells*—Demilune cells are the pallid remains of enormously distended erythrocytes As their name implies, they have the shape of the young crescent moon, sometimes with a thin circular line joining the horns of the crescent and continuing the shadow outline of the cell First described and named by the brothers Sergent (1905) whose original drawings are reproduced as figures 1 5 these corps en demilune are very common in blood films from malaria patients, so much so that they have been regarded as presumptive evidence of infection. Knowles and Senior-White (1927) believed them to be rather characteristic of vivax infection though Mary Lawson (1920) had described them in falciparum infection and Mayer (1908) had portrayed typical examples from the malaria infections of monkeys The plate shows the appearance of demilune cells in association with a falciparum gametocyte (*6*) a vivax segmenter (*7*), *and an unidentified trophozoite (8) The drawings were made from Malayan material An* extreme example of this peculiar distortion of the erythrocytes—a tracing from a microscopic field—is shown as figure 9, the film was taken from a Chinese baby with a light vivax infection and a very severe anaemia (red cells 1 14 million haemoglobin 3 8g /100 ml)

Though demilune cells are very common in blood films from malaria patients they may also appear when malaria can be excluded They are sometimes numerous in patients with severe anaemia (9), though anaemia is not necessarily the cause for we have seen them in blood *films from healthy persons The explanation of the phenomenon is unknown* That the demilune cells are artefacts is suggested by their greater frequency at the tail end of the spread where the cells have been rolled and tumbled along the slide The duration of fixation and staining of the films may also be important, for demilune cells are particularly common in films which have had a brief fixation and a prolonged staining

Pessary bodies—Another distinctive form of the damaged erythrocyte is the distorted form to which the brothers Sergent have given the name corps en pessaire The appearance of these corps en pessaire *is shown in figures 12 14, reproduced from the original drawings* of Sergent *et al* (1908) figures 10 and 11 show the presumed early stages in their formation

Pessary bodies are not uncommon in blood films from malaria patients (21 28) Like demilune cells they are probably artefacts produced during the preparation of the films and they tend similarly to be associated with anaemia They have no significance in malaria diagnosis Figures 15 20 portray the forms which the pessary bodies seem to arise The edges of a vacuole in the torn cell appear to be rolled back as a thickened darker staining margin *Finally, the remains of the erythrocytes take on the appearances shown in figures* 21 28

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* Als kn wn as gigantocytes (N b 1912) and corp scul skele n (Mary Laws n 9 0)

SERGENT ED AND SERGENT ET., (1905) Étud ép démi l giq es t p phylactiques d paludism n Algérie en 1904 *Ann Inst Pas ur 19* 1 9

KNOWLES R AND SENIOR WHITE R *M l n t m t g tion nd contr l* Thacker Spink & Co Calcutta 19 7

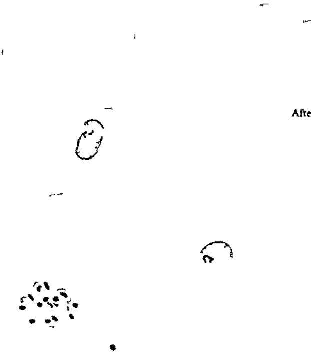
ROWLEY LAWSON MARY (1920) Cr nu bodie in sti o-autumnal malaria their migr ti and ttachment to the urface of the d corpuscl *J exp M d 31* 201

MAYER M. (1908) Übe Malari para t n b Affen *A h f P n t k nde 12* 314

SERGENT ED AND ET B UET M AND PLANTIER A. (1913) Ob ervations m croscop ques au cours d un accès pernicieux paludé n. *Bull S P t t 6* 6 5

NEEB H.M (1912) Some remarkabl paras tes in case of tertian malari *T an 2nd Congr Far Eastern A soc p M d* Hongko g *p* 306

XXXIV PUZZLES AND FALLACIES IN BLOOD EXAMINATION



After Sergent and Sergent 1905



After Sergent *et al* 1913 (10–14)

DEMILUNE CELLS AND PESSARY BODIES

XXXV PUZZLES AND FALLACIES IN BLOOD EXAMINATION*

Malaria diagnosis from stained blood films has pitfalls for the unwary Extraneous organisms from the skin, contaminants from the air, scratched, dirty, or defective slides deposits of stain, may all give rise to confusing artefacts and sometimes simulate malaria parasites Plates LVII to LIX portray some of the puzzling appearances found in Kuala Lumpur in the course of routine blood examinations for malaria This plate illustrates a few of the fallacies recorded by others So far as possible they are all reproduced at the same magnification

Yeasts and pollens (Figs 1-3)—Blood films exposed to the air in the course of field surveys may be contaminated by a variety of protozoal cysts, bacteria, fungi, yeasts and pollens, from the air Knowles *et al* (1929) have drawn attention to the possible errors from this source Figure 1 (yeast cells) and figures 2 3 (pollen grains), are reproduced from their detailed study of these and other sources of diagnostic fallacy They point out that yeasts may readily contaminate Giemsa stain which, from its richness in glycerine, encourages their growth We have not noted this tendency in Kuala Lumpur

X bodies—The curious bodies shown in figure 4 first described by Horrocks and Howell (1908) as X bodies, have received considerable attention Knowles *et al* (1929) regard them as semi crystalline deposits from old Leishman stain We do not recall having seen these bodies, possibly for the reason that we do not use Leishman stain by the conventional method The drawings are reproduced from Horrocks and Howell s original plate

Vegetable cells—Figures 5 10 assembled by Wenyon (1923) from various sources, portray bodies which have been found in blood films or spleen smears and mistaken for parasitic protozoa, the bodies are probably of vegetable origin

Fig 5 6—Vegetable cells described by Manson, 1905 (fig 5), and Bonsfield, *1911* (fig 6) from spleen smears taken from cases of kala azar

Fig 7—Vegetable cells and other organisms described by Elders (1911) as parasites in human blood

Fig 8—Vegetable cells described by Castellani and Willey (1905) from human blood

Fig 9—Vegetable cells found in blood films and described by *Dimond (1917) as the* cause of trench fever

Fig 10—Bodies which are probably vegetable cells found by Castellani (1914) in blood and spleen smears from a patient in Ceylon who had an enlarged spleen

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* *Th title f this plate is borrowed from B lfour (1911) who was among the firs t* clas ify and d cribe the common ource of error in th micro cop c examination of blood films in tr pical countri

BALFOUR A (1911) Fallacies and puzzl s n blood examination being an account of the more common deceptive appearances met with in uch work especially in tropical countri s *Fourth R port W ll om Tropical R sea h Labora ories V l A* Khartoum p 109

KNOWLES, R ACTON H W., AND DAS GUPTA B M (1929) Puzzles and fallacies in the examination of stain d films in the trop cs *I d n Med R Mem* N 13 Thacke Sp nk & Co Calcutta.

WENYON C M (1923) H emogregarines in man with not s on some other suppos d par s tes *T op Dis Bull 20* 527

HORROCKS W H AND HOWELL H A L. (908) X bodies f und in th blood f human b ing and nimals *J R Army med Cp 10* 351

MANSON P (1905) N tes on two cases of f bril trop cal splenom *galy (kala azar)* and a suggestion *Brit med J* 1261

BONSFIELD L. (1911) Remarks on kala azar in the Kassala and Blue Nile Distr cts of the Sudan. *Fourth Report W llcom Res a ch Lab tori* Khartoum Vol A p 138

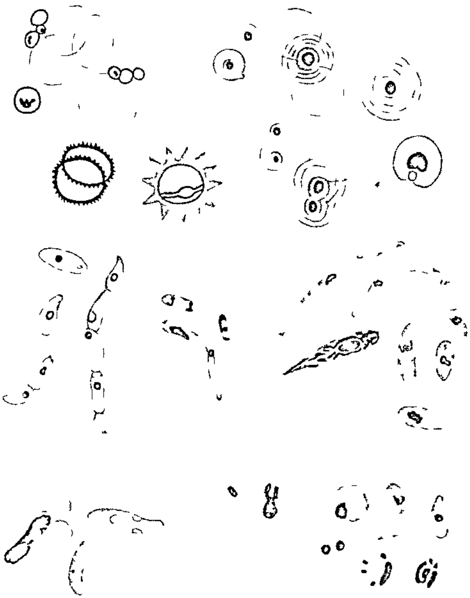
ELDERS C. (1911) U ber ine klini ch und ätiologi ch der Trypanosomias s und Schlafkrankheit *verwandt* Krankheit bei Javanen uf Sumatr *A ch Schiff -u. Tropen-Hyg 15* 1

CASTELLANI A AND WILLEY A (1905) Cit d by Wenyon (1923)

DIMOND L. (917) T e ch f r o P U O assoc ted with the prese c of a h em gr garin *Lanc t ii* 382

CASTELLANI A (1914) Not on certain protozo like bodi s in ca e of p otract d fev with splen *mega.y J t p Med H g 17* 113

XXXV PUZZLES AND FALLACIES IN BLOOD EXAMINATION



VARIOUS

XXXVI THE UNINFECTED ERYTHROCYTE

Aberrations in the morphology of the erythrocytes are common in disease, particularly in the anaemias The plate portrays the common appearances in stained films of normal abnormal, and distorted erythrocytes normoblasts and blood *pla elets* The drawings were made from films taken from malaria patients

Normal erythrocytes—The normal mature red cell (1) is a biconcave disc, some 7 8μ wide and 2 3μ thick The internal structure is not fully known, but the concept of an elastic cell with a thin limiting membrane, containing haemoglobin held as a fluid colloid is widely held

Variations in shape and size—Among the commonest red cell variations in malaria infections are the variations in shape (poikilocytosis) and size (anisocytosis) portrayed in *figures 2 and 3* The large purplish cell in figure 3 is a polychromatic macrocyte a large immature erythrocyte often seen in films from patients with malarial anaemia

Normoblasts—The erythrocytes are derived from nucleated parent cells known as *normoblasts* These cells are sometimes seen in blood films from patients with the grave forms of anaemia and rarely in the anaemia of severe and prolonged malaria A typical normoblast is shown in figure 4, figure 5 shows a basophilic normoblast with faint basophilic stippling

Howell Jolly bodies—The red cell inclusions known as Howell-Jolly bodies are thought to be the fragmentary remains of the nuclear chromatin (6-7) They are to be distinguished from the chromatin of degenerate or poorly stained parasites

Cabot s rings—Like Howell Jolly bodies, the loop or ring formations known as Cabot's rings have been regarded as the final remnants of the nucleus, but the work of Schleicher (1942) suggests that they are artefacts, configurations of denatured protein unrelated to the nucleus or nuclear membrane (9 12 14)

Diffuse basophilia, polychromasia—The immature erythrocyte still contains the remnants of a basophilic substance thought to be derived from the erythroblast (3 12 13) This substance gives to the stained cell a uniform bluish tinge (diffuse basophilia) often mingled in varied shades with the pale pink of the normal mature cell (polychromasia) These young basophilic or polychromatic erythrocytes are very common in blood films from malaria patients Rapid fixation of the films favours their appearance A polychromatic macrocyte with distorted Cabot s rings is shown in figure 12

Punctate basophilia—A basophilic stippling in some of the erythrocytes may occur in the severe anaemias, it is particularly common in lead poisoning and sometimes appears in films from malaria patients Typical examples are portrayed in figures 8 and 9

Reticulocytes—Reticulocytes are the immature erythrocytes which appear as poly chromatic cells in Romanowsky stained films (10-11) Certain dyes—of which brilliant cresyl blue is the best known—precipitate the basophilic substance of the immature cell The pre cipitated substance takes the dye to give the appearance of a bluish reticulum

Demilune cells and pessary bodies—Figures 15 18 (*see* also p 184)

Blood platelets—The platelets are non nucleated pale purple bodies usually about 2 3μ in diameter In unshed blood they appear to have a capsule but they disintegrate when the blood is shed and in stained films they have a characteristic woolly appearance with fuzzy edges They may be single (19) but often collect into clusters of half a dozen or more (23) Platelets of abnormal size or bizarre shape are not uncommon (21 22) A single platelet lying on a red cell (20) may be mistaken for a malaria parasite though the resemblance is rather remote

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Schleich r E M (1942) Th origin and natur of the Cab t ring bodies f rythr cytes *J lab lin Med* 27 983



THE ERYTHROCYTES AND THEIR VARIATIONS AND DISTORTIONS IN HEALTH AND DISEASE

XXXVII THE INFECTED ERYTHROCYTE

The invading malaria parasite often changes the form and appearance of the host erythrocyte These changes are related to the species of parasite and are extremely useful in diagnosis

Plasmodium falciparum (1 8)—The infected cells are unaltered in size but may show changes in colour and contour The vacuole of the parasite may be pale from the loss of haemoglobin in the cell beneath (2, 3 4) and cells containing large rings often have a purple tinge (4), usually associated with a thickened and darkened rim (3 4) Crenation of the host cell is common (3), even when the uninfected cells in the same field show no trace of this spiky distortion Maurer s spots are characteristic in cells containing the larger rings (3 4), they are of great diagnostic importance, but tend to lose their distinctive appearance at later stages (5) Cells containing young gametocytes do not show Maurer s spots (7) The characteristic bib of the mature gametocyte is derived from the host cell (8) The tendency to shrink and shrivel up described by the early Italian workers is seldom evident in the host cells of the Malayan parasite

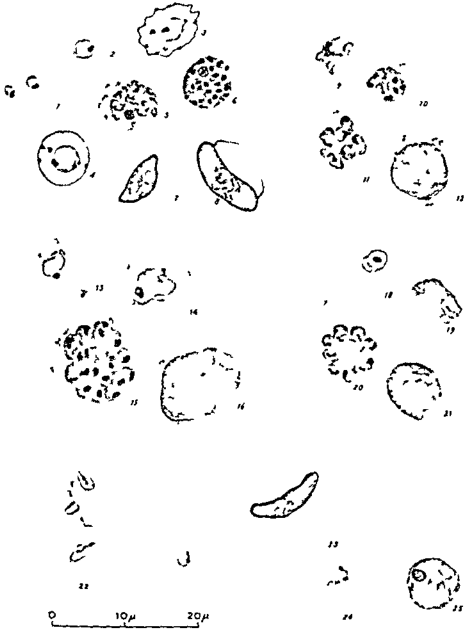
Plasmodium vivax (13 16)—The host cells enlarge at an early stage of invasion (13) the enlargement is progressive and cells containing mature parasites may have a diameter of 12μ or more (15 16) In fresh preparations the infected cells are pale, but the pallor is not obvious in stained films Nor is it usual to see a pallor in the vacuole of the parasite (cf 4 and 18) Schuffner s dots appear at a very early stage they are constant highly characteristic and extremely helpful in diagnosis (13 16) The dots increase in size and number with the growth of the organism but remain distinctive, unlike Maurer s spots they are present in cells containing gametocytes (16)

Plasmodium ovale (9 12)—The host cells are enlarged but the enlargement is less than that of vivax infected cells Schuffner s dots appear at an early stage and are constant in cells containing parasites which are advanced in growth The dots have a tendency to collect around the margin of the host cell (9) An elongation of the cell maybe with a fimbriation at one end is not uncommon (9)

Plasmodium malariae (18 21)—Erythrocytes invaded by *P malariae* show little change They are not enlarged—they may even be smaller than normal—and they show no general change in colour, though the vacuole may be pale from the loss of haemoglobin Ziemann s stippling is sometimes visible in the infected cells (18 19) but the dots are inconstant and have no diagnostic significance

Distortions of the host cells—Two fairly common distortions are illustrated in figures 22 25 In heavy falciparum infections the host cells tend to run together at the end of the spread particularly when the films are quickly dried cells and parasites are alike distorted (22) The grotesque distortion which finally appears as the demilune cell is portrayed in figures 23 25 a vacuole appears in the infected cell (24) the cell swells to several times the normal size (25) and finally it bursts to leave a pale staining crescentic residue (23) The examples shown are associated with a crescent (23) a young trophozoite of undetermined species (24) and a female vivax gametocyte (25) The presence of demilune cell in blood films was once regarded as presumptive evidence of vivax malaria But demilune cells are quite common in falciparum and malariae infections and even in blood films from persons who have never had malaria and their contribution to malaria diagnosis is not significant (*see* also p 184)

XXXVII THE INFECTED ERYTHROCYTE



THE CHANGES IN THE FORM AND STAINING OF THE MALARIA INFECTED CELL

XXXVIII ROMANOWSKY STAINING AND *p*H

The methods of staining blood films which are appropriate for routine haematological examinations are not always suitable for malaria diagnosis The haematologist is concerned mainly with the morphology and differentiation of the cellular elements of the blood the malariologist must specifically emphasise the staining of the parasites and the changes which they produce in the infected cells The difference in emphasis implies a difference in method The malariologist finds that his needs are best met in films which the haematologist might regard as over stained, and he must work within a narrower range of *p*H A beautiful differentiation of blood cells is possible in films stained at a *p*H of 6 8 or even lower, but at this level of *p*H the demonstration of stippling on the malaria infected cell is capricious The plate illustrates the influence of *p*H on the colour range of Romanowsky stained parasites and of their host cells Each group of four infected cells was drawn from films taken from the same patient and stained by the same method, the only difference was the *p*H of the diluent The stain was Leishman s, stabilised with glycerine used at a concentration of three drops for every cubic centimetre of buffer solution The films were stained for half an hour

Uninfected erythrocytes—The lower the *p*H, the paler and cleaner the staining Cells stained at a *p*H of 6 8 or less have a pale flesh colour Stained at a *p*H over 7 6 they appear darker, often with a purplish tint

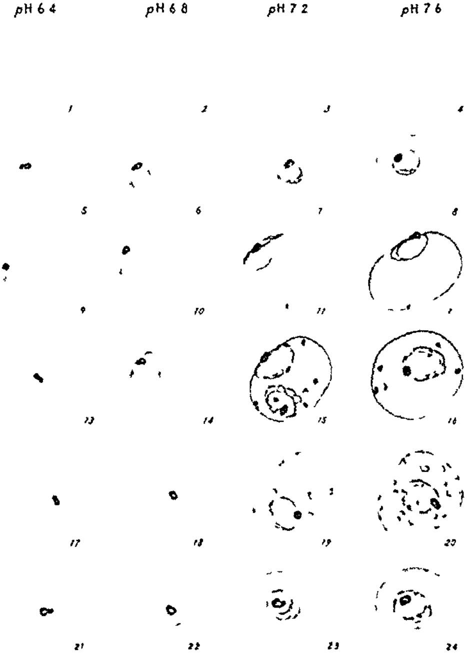
Chromatin—The nuclear chromatin of malaria parasites stains bright red at a *p*H 6.4 or less, ruby red or reddish purple at *p*H 7 2 and bluish purple at a *p*H over 7 6 The bright red chromatin of the parasites sometimes portrayed in colour drawings of the parasites suggests that the films were stained at a *p*H below 6 8

Cytoplasm—The staining of the cytoplasm of malaria parasites deepens *in intensity* as the *p*H rises stained at a *p*H of 6 0 it is almost invisible and at *p*H 6 4 it is still very pale The best definition is attained by staining at a *p*H between 7 2 and 7 6

Stippling of infected cells—The definition of Maurer s spots is sharply limited by the *p*H of the stain, they are invisible at *p*H 6 4 barely visible at *p*H 6 8, deeply stained at *p*H 7 2 and even more deeply at *p*H 7 6 Schuffner s dots are similarly though rather less sensitive to the *p*H of the stain They are pale but visible at *p*H 6 8, well defined at *p*H 7 2 and deeply stained at *p*H 7 6 Ziemann s dots can seldom be demonstrated at a *p*H below 7 0 The drawings illustrate these points

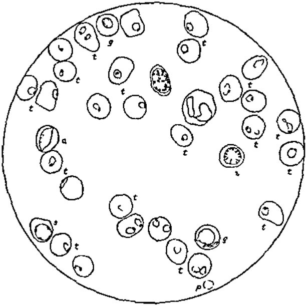
- Figs 1 4 Uninfected erythrocytes
- Figs 5 8 Erythrocytes containing young falciparum rings, Maurer s spots are not found at this stage
- Figs 9 12 Slightly older falciparum rings early Maurer s spots just visible at *p*H 6 8
- Figs 13 16 Still older falciparum rings developed Maurer's spots *are just visible* at *p*H 6 8
- Figs 17 20 Young vivax rings Schuffner s dots just visible at *p*H 6 8
- Figs 21 24 Young quartan rings Ziemann s dots are first visible at *p*H 7.2

Distilled water is usually acid in reaction even when fresh For Romanowsky staining the reaction may be adjusted to neutrality by the addition of alkali more simply, a stable *p*H around neutrality may be maintained by the use of a phosphate buffer solution as a diluent for the stain



THE COLOUR AND DEFINITION OF THE NUCLEAR CHROMATIN AND CYTOPLASM OF THE MALARIA PARASITES IN RELATION TO THE *p*H OF LEISHMAN STAIN

XXXIX *Plasmodium falciparum*—FATAL INFECTION

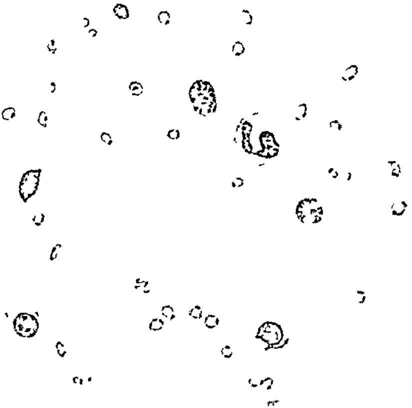


Original field with the addition of a few parasites drawn from neighbouring fields and indicated in the key by roman lettering

t—young ring trophozoites
t[1]—tenue form trophozoites
s[1]—early schizont
s —advanced schizonts
g[1]—very young intracorpuscular gametocyte
g —young oat shaped intracorpuscular gametocyte
g —young intracorpuscular gametocyte with Garnham body in the infected cell
n—neutrophil polymorph
p—blood platelet

Clinical note—The patient a Chinese dhoby was admitted to the General Hospital Seremban during the morning of 31st March 195_ with a history of 4 days fever and a temperature of 103 F The patient was conscious but could not speak he lapsed into coma and died at 5 30 p m on the same day There was no autopsy examination The parasite density was not determined, a thin film count showed that 25 per cent of the red cells were infected (We are indebted to Dr J E MacMahon for the clinical note and to Mr I G Spittel for the film)

XXXIX *Plasmodium falciparum*

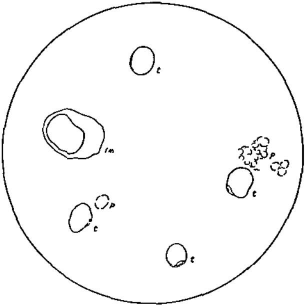


FATAL INFECTION

The drawing was made from a film taken three hours before death Most of the parasites were young rings in cells as yet unstippled with Maurer s spots Th high parasite density and the presence of schizonts and young gam to ytes indicate th gravity of the infection. Garnham bodies were present in some of the cells invaded by young gametocytes

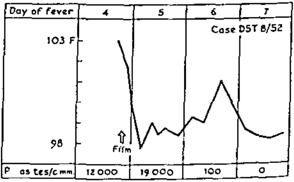
XL MARGINAL TROPHOZOITES

Original field

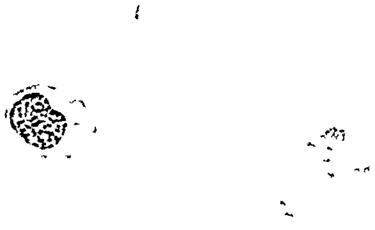


t—marginal trophozoites
lm—large mononuclear leucocyte
p—blood platelets

Clinical note—The patient an Indian boy was admitted to the General Hospital Kuala Lumpur on January 3rd 1952 with a mild falciparum infection Treatment with Daraprim began on the 4th day of fever



XL *Plasmodium falciparum*

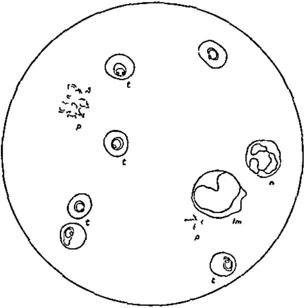


MARGINAL TROPHOZOITES

A marginal attachment of the trophozoites of *Plasmodium falciparum* is fairly common. These marginal forms, also known as accolé forms, are found alike in mild and severe infections. Their significance lies mainly in the chance that they may be overlooked in routine blood examination. The smallest forms, each draped as a thin line along the margin of the host cell, are easily missed or mistaken for artefacts. The drawing was made from a blood film in which nearly eighty per cent. of the parasites had a marginal position (*see* also plate V).

XLI *Plasmodium falciparum*—LARGE RING TROPHOZOITES

Original field



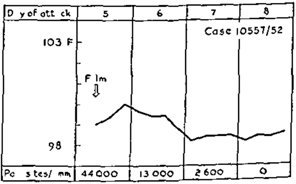
t—large trophozoites in cells which are stippled with Maurer's spots, a purplish rim is visible in some of the host cells but the purple tinge which is not uncommon in the invaded cell at this stage is not shown in the plate

n—neutrophil polymorph

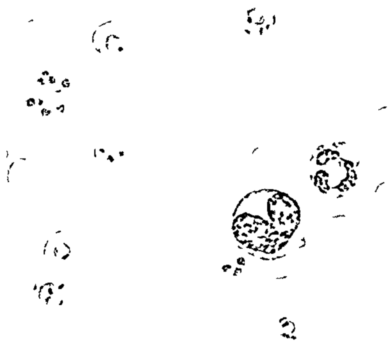
lm—large mononuclear leucocyte

p—blood platelets

Clinical note—The patient a Malay policeman was admitted with a mild falciparum infection to the General Hospital, Kuala Lumpur on September 17th 1952 Treatment with Paludrine began on the 5th day of fever At noon on the day of admission 92 per cent of the rings were large at 9 p m 80 per cent were very small (We are indebted to Dr T Wilson for the film and clinical note)



XLI *Plasmodium falciparum*

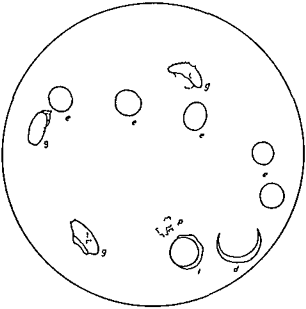


LARGE RING TROPHOZOITES

This is the growth phase at which falciparum trophozoites normally leave the peripheral circulation to stagnate in the capillaries of the internal organs The parasites are large and fleshy rings the host cells are stippled with Maurer s spots Often the host cells have a purple tinge and a thickened purple rim Blood films from patients with falciparum infections which are dominated by a single large brood show these large rings in the fever free interval between paroxysms but the simultaneous development of several broods is usual in Malayan infections and the trophozoites found in blood films at any one time are then of mixed phase The drawing was made from a film taken on the fifth day of fever from a patient with a mild infection The possible relationship between ring size and immunity is discussed in the text

XLII *Plasmodium falciparum*—ADULT GAMETOCYTES

Original field

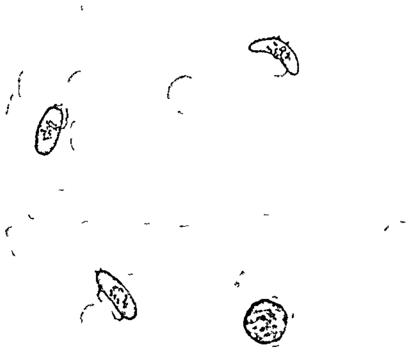


g¹—adult female gametocytes
g —adult male gametocyte
d—demi lune erythrocyte
e¹—polychromatic erythrocytes
c —polychromatic erythrocyte with slight punctate basophilia
l—lymphocyte
p—blood platelets

Clinical note—The patient, a Chinese woman 8 months pregnant was admitted to the Tampin Hospital on May 12th 1952 with a history of nine days of fever She was afebrile on admission and remained afebrile throughout her stay in hospital Blood examination revealed a mild falciparum infection The daily parasite counts per c mm of blood during the first five days of Nivaquine treatment are summarised below The blood film was taken on the 13th day (We are indebted to Dr J F B Edeson for the film and the clinical note)

Alleged day of attack	9	10	11	12	13
Trophozoites per c mm	30 000	5 700	300	100	0
Gametocytes per c mm	1 300	10 000	8 600	6,700	11 600

XLII *Plasmodium falciparum*

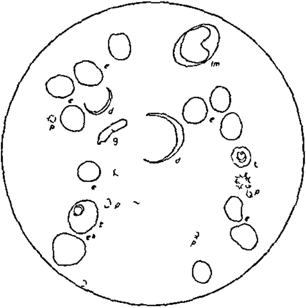


ADULT GAMETOCYTES

The adult gametocytes of *P falciparum* the well known crescents appear in the peripheral blood for the first time rather late in the attack usually after about ten days of fever They are then full grown as are those shown in the plate In the absence of gametocytocidal treatment they tend to increase in numbers for a few days and then to diminish and disappear over the next two or three weeks The young forms develop in the bone marrow and are found in films from the finger blood only in heavy infections The film was taken on the thirteenth day of the attack when the patient had been free from fever for at least five days

XLIII *Plasmodium falciparum*—MALARIAL ANAEMIA

Original field



t—falciparum trophozoites in cells stippled with Maurer s spots one infected cell is a polychromatic macrocyte

g—adult female gametocyte

e¹—polychromatic erythrocytes

e —polychromatic macrocytes

d—*demilune erythrocytes*

lm—large mononuclear leucocyte

p—blood platelets

Clinical note—The patient, an Indian infant one year old was admitted to the General Hospital, Kuala Lumpur on October 9th 1952, with a primary falciparum infection The child was said to have had a week of fever, but the presence of adult cresents in blood films suggested a longer duration In hospital the child had no fever after the first day The spleen was two fingers enlarged and there was oedema of the ankles The blood film was taken soon after admission The haematological reports at this time and twelve days later were

	11 10 1952	23 10 1952
Haemoglobin	6 3 gm /100 ml blood	8 7 gm /100 ml
Erythrocytes	3 2 million	4 8 million

(We are indebted to Dr I H Turner for the clinical note)

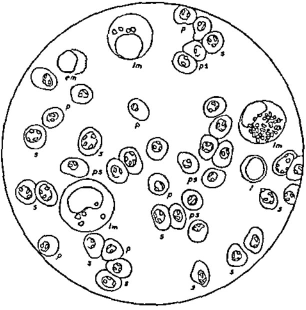
XLIII *Plasmodium falciparum*



MALARIAL ANAEMIA

Malaria infections are always associated with a destruction of erythrocytes The losses are quickly restored in mild attacks of brief duration but the acute or cumulative loss of erythrocytes in severe or prolonged attacks is beyond the capacity of the host for replacement with anaemia as a result The parasite density in serious falciparum infections may exceed half a million per cubic millimetre of blood and a reduction of the erythrocytes in untreated primary attacks to two million per cubic millimetre or less is not uncommon The anaemia tends to be of a simple iron-deficiency type and recovery is usual with no treatment beyond a good diet and supplementary iron during convalescence The blood film taken from an Indian infant at a late stage of a primary falciparum attack portrays appearances which are very common in severe falciparum infections The main features of the blood picture are the anisocytosis and the many polychromatic erythrocytes

XLIV *Plasmodium falciparum*—PLACENTAL INFECTION



Original field

ps—presegmenters

s^1—early schizonts

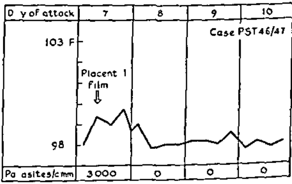
s —late schizont

lm—large mononuclear leucocytes with phagocytosed masses of malarial pigment each mass is derived from a mature falciparum schizont

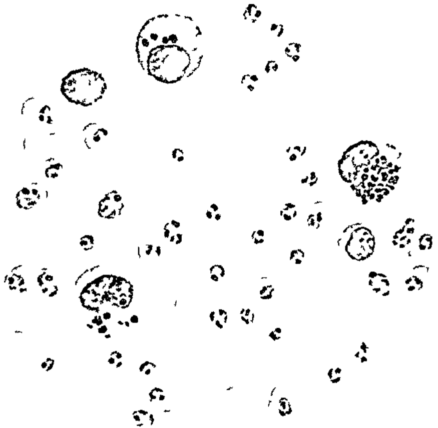
l—lymphocyte

em—eosinophil myelocyte

Clinical note—The patient, an Indian woman, was admitted in labour to the Tampin Hospital on May 9th 1947 She had slight fever Blood examination revealed a very light falciparum infection The parasites disappeared from the maternal blood films after a single dose of Paludrine (We are indebted to Dr J F B Edeson for the film and clinical note)



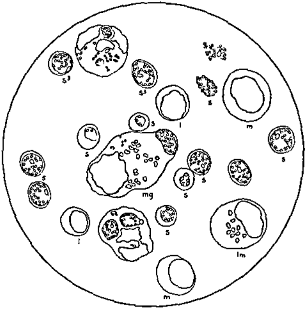
XLIV *Plasmodium falciparum*



PLACENTAL INFECTION

The tendency for *Plasmodium falciparum* to stagnate in the intervillous spaces of the placenta is well known Enormous accumulations of segmenting parasites are sometimes seen in placental films even when the maternal infection is light The drawing was made from a film taken from the placenta of an Indian woman with a very mild infection Most of the parasites are presegmenters or early segmenters The pigment of the parasites is concentrated into a single solid mass, the nuclear chromatin is rather indistinct as is usual at this stage The absence of Maurer s spots in the infected cells is probably attributable to fading—the film was stained in 1947 and the microscopic field was drawn in 1952

XLV *Plasmodium falciparum*—PHAGOCYTOSIS



Composite field an assembly of parasites and cells drawn from the same placental film

s^1—early schizonts

s —advanced schizonts

s^3—mature schizonts

s^4—ruptured segmenter

n—neutrophil polymorphs, one contains two schizonts and a single mass of pigment from a third schizont, and in the cytoplasm of the second may be seen two early schizonts and many merozoites Both polymorphs are swollen and rather degenerate

lm—large mononuclear leucocyte with ten masses of pigment in the cytoplasm

mg—large macrophage cell with evidence of extreme phagocytic activity The cell is vacuolated and rather degenerate The cytoplasm contains a mature schizont and numerous masses of pigment each derived from a schizont The source of the small granules of pigment whether from a gametocyte or from trophozoites is uncertain

l—lymphocytes

m—neutrophil myelocytes

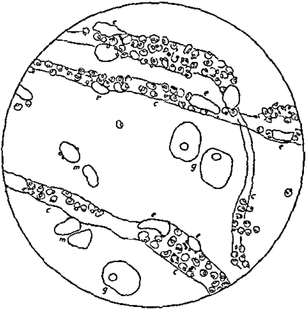
XLV *Plasmodium falciparum*



PHAGOCYTOSIS

The destruction of the parasites by phagocytic cells is an important mechanism of defence in malaria infections Evidence of phagocytosis may be observed in films from the finger blood especially in severe falciparum infections but the main arena seems to be the spleen where the macrophage cells derived from the reticulo-endothelial system circulate sluggishly in the sinusoids and take a heavy toll of the parasites There is active phagocytosis too, in the bone marrow in the liver and in pregnant women in the placenta The cells illustrated in the plate were drawn from a placental film.

XLVI *Plasmodium falciparum*—CEREBRAL INFECTION



Original field
Colour correction—the 'reds' are over emphasised

c—capillaries packed with parasites at a pre segmenting or early segmenting stage some of the parasites lie outside the capillaries, possibly from displacement during the spreading of the smear Each parasite has a prominent mass of pigment, it is this pigment which is so obvious in fresh 'squash' preparations of brain as dark dots ranged along the lines of the capillaries

e—nuclei of the capillary endothelial cells

g—nuclei of glial cells

m—nuclei of glial or microglial cells

Clinical note—The patient, a Chinese woman 38 years old was admitted to the District Hospital Tampin in deep coma on May 1st 1952 Blood examination revealed an extremely heavy falciparum infection with 430,000 parasites per c mm of blood The patient died a few *hours later There was no full post mortem examination The smear* was prepared from material obtained 18 hours after death by a needle autopsy through the orbit (We are indebted to Dr J F B Edeson for the brain smear and clinical note)

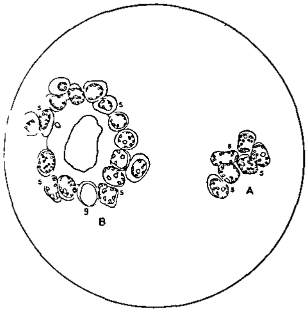
XLVI *Plasmodium falciparum*



CEREBRAL INFECTION

When a patient with falciparum malaria dies in coma the brain capillaries may be packed tight with parasites and it is not illogical to assume that anoxia from the arrest of the capillary circulation may have been the cause of death The drawing was made from the brain of a Chinese woman who was admitted to hospital in deep coma She had a very heavy falciparum infection and died a few hours later The cerebral capillaries at the base of the brain were distended with great accumulations of parasites mostly presegmenters

XLVII *Plasmodium falciparum*—CULTURE



Composite field the two clusters were drawn from neighbouring fields of the same film

A—cluster of segmenting parasites agglutination of the infected cells in this particular culture was first observed when the parasites had reached the pre segmenting stage, seven hours after the blood was drawn

B—apparent attraction of segmenting parasites towards a large mononuclear leucocyte

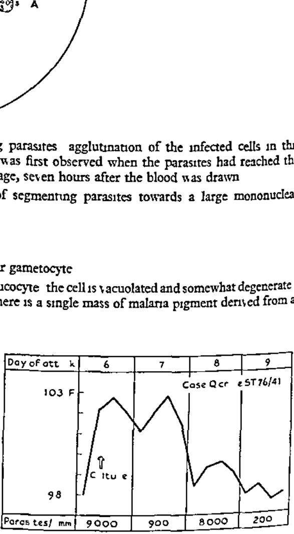
s¹—early schizonts

s —advanced schizonts

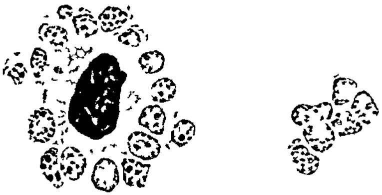
g—young intracorpuscular gametocyte

lm—large mononuclear leucocyte the cell is vacuolated and somewhat degenerate in the cytoplasm there is a single mass of malaria pigment derived from a mature schizont

Clinical note—The patient an Indian adult, was admitted to the General Hospital Kuala Lumpur on 30th November 1941 with a mild falciparum infection Blood was drawn before the start of quinacrine treatment, the parasites were then large rings 18 24 hours old The cultures were made by the Bass technique the film was prepared from the 24 hour culture



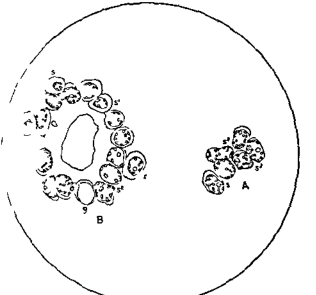
XLVII *Plasmodium falciparum*



CULTURE

When malaria infected blood is withdrawn from the body the parasites usually die but they survive for a time and even continue to grow in defibrinated blood to which a little glucose has been added The young trophozoites may reach maturity but there are heavy losses during segmentation and special methods are necessary to promote the continued development of the parasites beyond the first generation A prolonged maintenance in cultures through many asexual generations has not been achieved in the species affecting man The film prepared from a 24 hour culture illustrates two well known phenomena—the agglutination of infected cells into clusters and the remarkable adherence of these cells to the large mononuclear leucocytes The clusters of parasites are sometimes big enough to fill the high power field of the microscope circulating as emboli and becoming impacted in the capillaries of vital organs they have been held as a possible cause of pernicious forms of infection The apparent attraction of segmenting parasites towards the large mononuclears may perhaps be related to the marked phagocytic activity of these cells

XLVII *Plasmodium falciparum*—CULTURE



Composite field the two clusters were drawn from neighbouring fields of the same film

A—*cluster of* segmenting parasites agglutination of the infected cells in this particular culture was first observed when the parasites had reached the pre-segmenting stage, seven hours after the blood was drawn

B—apparent attraction of segmenting parasites towards a large mononuclear leucocyte

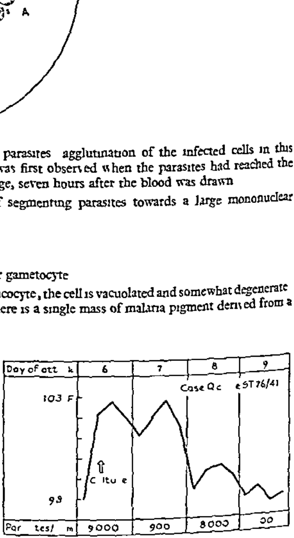
s'—early schizonts

s —advanced schizonts

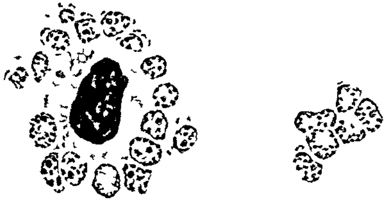
g—young intracorpuscular gametocyte

lm—large mononuclear leucocyte, the cell is vacuolated and somewhat degenerate in the cytoplasm there is a single mass of malaria pigment derived from a mature schizont

Clinical note—The patient an Indian adult was admitted to the General Hospital Kuala Lumpur on 30th November 1941 with a mild falciparum infection Blood was drawn before the start of quinacrine treatment the parasites were then large rings 18 24 hours old The cultures were made by the Bass technique the film was prepared from the 24 hour culture



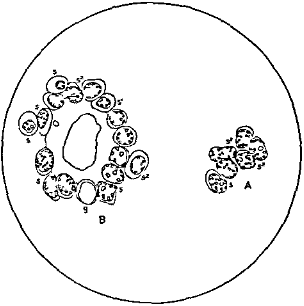
XLVII *Plasmodium falciparum*



CULTURE

When malaria infected blood is withdrawn from the body the parasites usually die but they survive for a time and even continue to grow in defibrinated blood to which a little glucose has been added The young trophozoites may reach maturity but there are heavy losses during segmentation and special methods are necessary to promote the continued development of the parasites beyond the first generation A prolonged maintenance in cultures through many asexual generations has not been achieved in the species affecting man The film prepared from a 24 hour culture illustrates two well known phenomena—the agglutination of infected cells into clusters and the remarkable adherence of these cells to the large mononuclear leucocytes The clusters of parasites are sometimes big enough to fill the high power field of the microscope circulating as emboli and becoming impacted in the capillaries of vital organs they have been held as a possible cause of pernicious forms of infection The apparent attraction of segmenting parasites towards the large mononuclears may perhaps be related to the marked phagocytic activity of these cells

XLVII *Plasmodium falciparum*—CULTURE



Composite field the two clusters were drawn from neighbouring fields of the same film.

A—cluster of segmenting parasites agglutination of the infected cells in this particular culture was first observed when the parasites had reached the pre-segmenting stage, seven hours after the blood was drawn.

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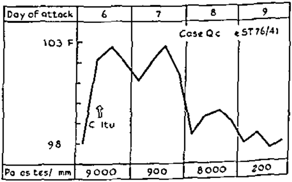
s¹—early schizonts

s²—advanced schizonts

g—young intracorpuscular gametocyte

lm—large mononuclear leucocyte, the cell is vacuolated and somewhat degenerate, in the cytoplasm there is a single mass of malaria pigment derived from a mature schizont

Clinical note—The patient an Indian adult was admitted to the General Hospital Kuala Lumpur on 30th November 1941 with a mild falciparum infection Blood was drawn before the start of quinacrine treatment, the parasites were then large rings 18 24 hours old The cultures were made by the Bass technique the film was prepared from the 24 hour culture



XLVIII *Plasmodium falciparum*

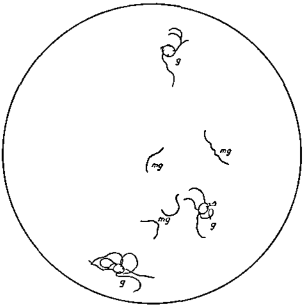


EXFLAGELLATION OF THE MALE GAMETOCYTES

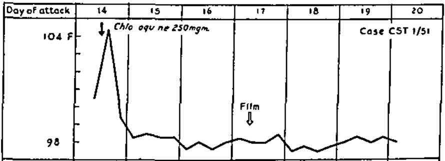
The formation and liberation from the male gametocyte of the male gametes the phenomenon known somewhat inappropriately as exflagellation normally occurs in the midgut of the mosquito The process is never seen in thin blood films prepared for routine malaria diagnosis but may be demonstrated in films which have been kept in a moist chamber and prevented from drying The drawing was made from a thin film taken on the seventeenth day of a falciparum attack from a patient with many gametocytes in the blood Many of the gametes are still attached to the parent gametocyte but some have broken free The apparent absence of nuclear chromatin from the gametes suggests that they are sterile, the gametes may sometimes break away before they have received their quota of nuclear material

XLVIII *Plasmodium falciparum*—EXFLAGELLATION OF THE MALE GAMETOCYTES

Original field



g—exflagellating male gametocytes
mg—male gametes



Day of attack	14	15	16	17	18	19	20
Ri gs/c mm	300	5 800	400	0	0	0	0
C esc ts/c mm	3100	4 400	4 800	13 600	13 100	10 200	14 600

The patient, an Indian adult was admitted to the General Hospital Kuala Lumpur on 31st October, 1951, with a mild falciparum infection The films taken seventeen days after the start of the attack at a time when there were many gametocytes in the blood were prepared and stained by the method described on p 104 The drawing was made from a film which had been kept for six minutes in a moist chamber (We are indebted to Dr T Wilson for the film and clinical note)

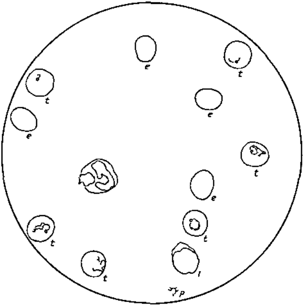
XLIX *Plasmodium vivax*



EARLY TROPHOZOITES

The plate illustrates the appearance of the parasites in blood films at the height of the vivax paroxysm or a little later The infection was due to a single rather straggling brood and the parasites were nearly all young trophozoites Enlargement of the host cell began very early and Schuffner s dots were present in most of the enlarged infected cells as is usual in vivax infections of Malayan origin The drawing was made from a film taken three hours after the start of the rigor and already more than a half of the cells containing ring forms were enlarged and ninety eight per cent contained Schuffner s dots two hours later all the infected cells were enlarged and stippled

XLIX *Plasmodium vivax*—EARLY TROPHOZOITES



Original field

t—young trophozoites, the host cells are already enlarged with a light sprinkling of Schuffner's dots the parasites are probably about three hours old

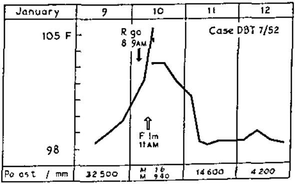
t[1]—young trophozoites in poly chromatic host cell the vacuole of the parasite is decolourised

e—polychromatic erythrocytes

n—neutrophil polymorph

l—lymphocyte

Clinical note—The patient a Malay policeman was admitted to the General Hospital Kuala Lumpur on 9th January 1952 with a history of an irregularly relapsing fever The spleen was moderately enlarged There was no fever on the day of admission but a sharp attack the next morning when the film was taken Daraprim treatment began on January 10th



Parasitological note—The table indicates the percentages of parasites at each stage of growth

	8 a m	9 30 a m	11 a m	12 30 p m	2 p m
Early trophozoites (rings)	47	80	90	95	96
Late trophozoites (amoeboids)	5	0	1	0	0
Schizonts	44	20	8	3	2
Gametocytes	3	0	1	1	2

Although the ring forms in the film were little more than 3 hours old 56 per cent of the host cells were enlarged and 98 per cent were stippled with Schuffner's dots (We are indebted to Dr T Wilson for the films and clinical note)

XLIX *Plasmodium vivax*

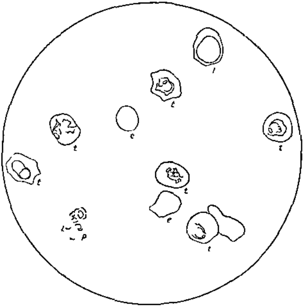


EARLY TROPHOZOITES

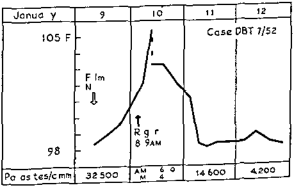
The plate illustrates the appearance of the parasites in blood films at the height of the vivax paroxysm or a little later The infection was due to a single rather straggling brood and the parasites were nearly all young trophozoites Enlargement of the host cell began very early and Schuffner s dots were present in most of the enlarged infected cells as is usual in vivax infections of Malayan origin The drawing was made from a film taken three hours after the start of the rigor and already more than a half of the cells containing ring forms were enlarged and ninety eight per cent contained Schuffner s dots two hours later all the infected cells were enlarged and stippled

L *Plasmodium vivax*—ADVANCED TROPHOZOITES

Original field



t—advanced trophozoites in enlarged cells stippled with Schuffner s dots
e—polychromatic erythrocytes
l—lymphocyte
p—blood platelets



The patient was a Malay police man with a relapsing vivax infection (*see* p 214 for clinical note)

Parasitological note—All the in fected cells seen in the film were en larged and stippled with Schuffner s dots The table indicates the percent ages of parasites at each stage of growth

	Noon	6 p m	10 p m
Early trophozoites (rings)	2	1	0
Advanced trophozoites (amoeboids)	95	94	9-
Schizonts	0	0	0
Gametocytes	3	5	8

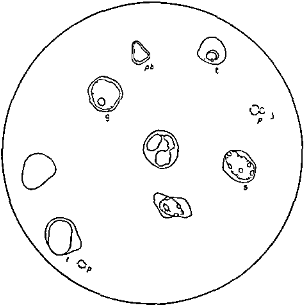
L *Plasmodium vivax*



ADVANCED TROPHOZOITES

When vivax infections produce a tertian fever the parasites found in blood films on the fever free day are advanced trophozoites The drawing was made from a film taken twenty hours before the start of the rigor nearly all the parasites on the film were at about the stage shown in the plate

LI *Plasmodium vivax*—VARIOUS FORMS

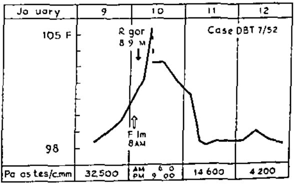


Original field
Colour correction—erythrocytes too yellow

- t—young ring form trophozoite in an enlarged polychromatic cell, the cell was stippled with purplish dots resembling those of punctate basophilia
- s—immature schizonts
- g—adult female gametocyte
- e—polychromatic macrocyte
- pb—pessary body (corps en pessaire)
- n—neutrophil polymorph
- l—lymphocyte
- p—blood platelets

Clinical note—The patient was a Malay policeman with a relapsing vivax infection (*see* p 214 for clinical note)

Parasitological note—The parasites were mostly very young forms and schizonts We suspect that the initial invasion of some of the host cells may have taken place in the capillaries of the internal organs, although many of the rings had but newly appeared in the peripheral blood, 39 per cent of their host cells were already enlarged and 79 per cent were stippled with Schuffner s dots The percentage of parasites at each stage of growth is shown in the table



	9th	10th		
	10 p m	8 a m	11 a m	2 p m
Early trophozoites (rings)	0	47	90	96
Advanced trophozoites (amoeboids)	92	5	1	0
Schizonts	0	44	8	-
Gametocytes	8	3	1	-

LI *Plasmodium vivax*

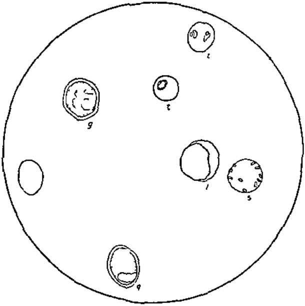


VARIOUS FORMS

The drawing was made from a blood film taken at the beginning of a rigor in a relapsing vivax infection Ring forms of the new generation had appeared but there were still schizonts of the earlier generation in considerable numbers A few gametocytes were also present in the film Six hours later there were still a few schizonts indicating a considerable lag in the maturing of the particular generation Most of the ring forms were found in cells already stippled with Schuffner s dots four hours later all infected cells were stippled

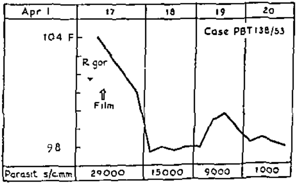
LII *Plasmodium vivax*—VARIOUS FORMS

Original field



t—young ring form trophozoites in host cells which show early Schuffner's dots but little enlargement
s—advanced schizont
g¹—adult male gametocyte
g²—adult female gametocyte
e—polychromatic erythrocyte
l—lymphocyte

Clinical note—The patient a Malay soldier, was admitted on 17th April 1953 to the District Hospital Tampin, on the first day of a vivax attack—possibly a relapse as he had had a similar attack six months before The patient stated that he had been taking 100 mg Paludrine daily He had high fever on admission at 8 a m and is said to have had a rigor shortly before Paludrine treatment began on the first day of fever

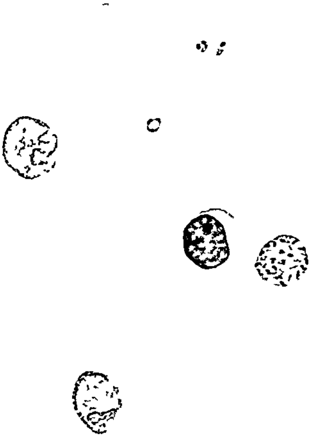


Parasitological note—The parasites present in the film were

Early trophozoites (rings)	89 per cent
Advanced trophozoites (amoeboids)	1
Schizonts	1
Gametocytes	9

Schuffner's dots were present in 84 per cent of the cells containing young rings and 69 per cent of these cells were larger than normal (We are indebted to Dr J F B Edeson for the film and clinical note)

III *Plasmodium vivax*

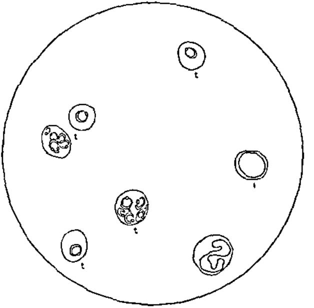


VARIOUS FORMS

Blood films taken at the height of the vivax paroxysm usually show young ring forms and possibly a few segmenters and gametocytes When the fever is tertian from the maturing every forty-eight hours of a single generation nearly all the parasites are likely to be at this stage or phase The plate portrays the typical appearances The film was taken on the first day of a vivax attack about two hours after a rigor

When the fever is quotidian from the segmentation on alternate days of two broods blood films taken at the height of the fever will also reveal half grown parasites of the generation due to segment the next day This development of *P vivax* in two broods about twenty four hours apart is very common. Why the number of broods should so often be restricted to two and why the broods should be so evenly spaced is not known.

LIII *Plasmodium vivax*—MULTIPLE INFECTION OF CELLS



Composite field—the multiple infected cells were drawn from different fields of the same film

t—young trophozoites
n—neutrophil polymorph
l—lymphocyte

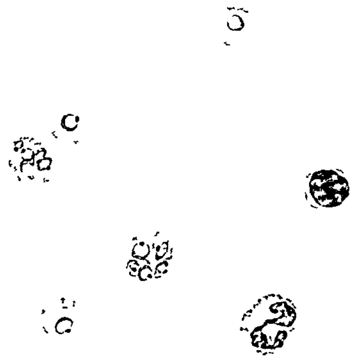
Clinical note—The patient, an elderly Cantonese was admitted to the General Hospital Kuala Lumpur on February 1st 1940 with an acute vivax infection He had had fever for five days before admission, but could not recall having had any previous fever within twelve months Clinically the case was unusual in the low fever and the exceptionally heavy infection The patient was not very ill—at no time in hospital did the temperature exceed 100 F —and he made an uneventful recovery on treatment with quinine

Parasitological note—The blood film taken on the seventh day of fever revealed an extra ordinary parasitological picture The parasite density exceeded 100 000 per c mm of blood all stages of *P vivax* were present but mainly young ring forms The advanced trophozoites schizonts and gametocytes were morphologically typical, but the young trophozoites showed an extraordinary tendency to crowd into particular cells Many cells contained four, five or six

	Parasites per cell						
	1	2	3	4	5	6	6+
Infected cells	312	99	40	35	9	4	1
Percentage	62	20	8	7	2	1	—

parasites, one—not shown in the plate—appeared to have eight The table indicates the number of rings in 500 infected cells

LIII *Plasmodium vivax*



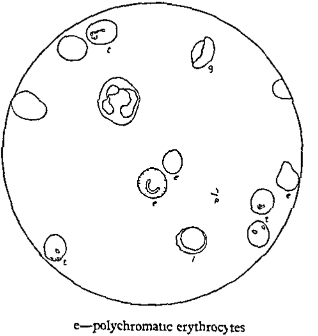
MULTIPLE INFECTION OF CELLS

The invasion of a single erythrocyte by two or more merozoites occurs in all forms of malaria but with a differing frequency The occurrence is so common in falciparum malaria as to have a minor diagnostic significance in vivax infections the presence of more than three rings in a cell is unusual and in quartan infections we have never seen more than two The phenomenon is related to the random odds of distribution in heavy infections but other factors may be involved

A marked tendency to multiple infection of the host cells in vivax infection is known but the phenomenon is so rare that the circumstances have sometimes been placed on record The plate illustrates the appearances of the parasites in one of these rare infections

LIV MIXED VIVAX AND FALCIPARUM INFECTION MALARIAL ANAEMIA

Original field



t—vivax trophozoites in cells heavily stippled with Schuffner s dots little enlargement in three of the cells, the parasites appear to be rather degenerate

g—falciparum gametocyte

e—polychromatic erythrocytes

e[1]—polychromatic erythrocyte with punctate basophilia and a cleft in the cell

n—neutrophil polymorph

l—lymphocyte

p—blood platelet

Clinical note—The patient a Malay fisherman was admitted to the General Hospital Kuala Lumpur, on December 8th 1952, with a history of irregular fever for one month His spleen was slightly enlarged and he was very anaemic Blood examination revealed a mixed vivax and falciparum infection, the parasite density was not recorded There was considerable achromasia but the central pallor in the erythrocytes is not well shown in the plate Treatment with Paludrine began on the day of admission, the temperature fell to normal within three days and asexual parasites disappeared from blood films within five days The anaemia was treated with a simple ferrous sulphate preparation The haemoglobin and erythrocyte concentrations during ten weeks observation in hospital are summarised in the table

	Day 0*	8	14	22	28	4-	70
Haemoglobin	30% (Sahli)	5 5 g /100 ml	6 8 g	7 8 g	9 1 g	13 2 g	15 1 g
Erythrocytes (millions)	2 2	3 2	4 0	4 2	5 9	6 2	6 5

* Day of adm n to ho p t l

Parasitological note—The film showed *P vivax* and *P falciparum* in the proportions—vivax trophozoites 95, vivax gametocytes 4 falciparum gametocytes 1 More than half of the vivax infected cells appeared to show little or no enlargement (We are indebted to Dr *L H Turner* for the clinical note and to Miss Elizabeth Cheek for the blood estimations)

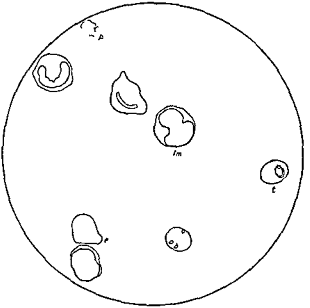
LIV MIXED VIVAX AND FALCIPARUM INFECTION



MALARIAL ANAEMIA

Abnormal appearances in *Plasmodium vivax* are not uncommon in anaemic patients with prolonged fever from untreated infection The host cells may show little enlargement and the parasites under these circumstances may sometimes acquire an ovale like morphology The drawing was made from a film taken from an anaemic Malay patient with a mixed vivax and falciparum infection of one month s duration The vivax infected cells were heavily supplied with Schuffner s dots but many of the cells were but little enlarged

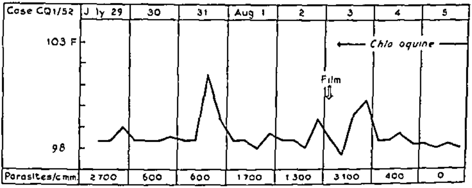
LV *Plasmodium malariae*



Original field

t—young trophozoite
s—mature schizont
e—polychromatic macrocytes, one distorted with a cleft down the middle
n—neutrophil polymorph
l—lymphocyte
lm—large mononuclear leucocyte
p—blood platelets

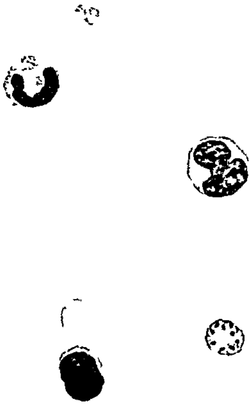
Clinical note—The patient an Indian woman 30 years old was admitted for confinement to the District Hospital Tampin, on July 29th 1952 She said that she had been feverish for five days but she was afebrile on admission to hospital A month before she had had a similar



attack The spleen was not palpable There was a vague history of Paludrine treatment Blood examination revealed a low grade quartan infection with evidence of a macrocytic anaemia The haemoglobin reading was 40 per cent (Tallqvist) and a few normoblasts were present in the film The large size of the erythrocytes will be clear from a comparison of the plate with earlier plates all drawn to the same scale

Parasitological note—The film was taken on the morning of 3rd August when the patient though afebrile at the time was restless, with a cold clammy skin The parasites present were young rings advanced trophozoites segmenters and gametocytes in the proportions 9 46 36 9, the rings and schizonts represented the rather straggling brood responsible for a mild paroxysm a few hours after the film was taken, the older trophozoites arose from a generation which had segmented and produced a slight rise in temperature the day before (We are indebted to Dr J F B Edeson for the film and the clinical note)

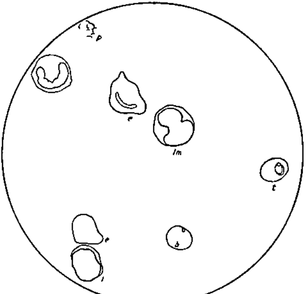
LV *Plasmodium malariae*



QUARTAN MALARIA WITH MACROCYTIC ANAEMIA

The parasite density in quartan infections is usually low The count seldom exceeds 10 000 per c mm of blood and lower counts are usual Many patients are afebrile in the later stages of infection and the parasites are discovered by chance in the course of routine blood examination The plate shows a mature schizont and a young trophozoite a combination which is usual at the time of the paroxysm in patients with a quartan temperature The drawing was made from an Indian woman with a low grade infection She was pregnant and had been admitted to hospital for confinement The blood film revealed a rather marked macrocytosis which we suspect to have been related more to the pregnancy than to the malaria

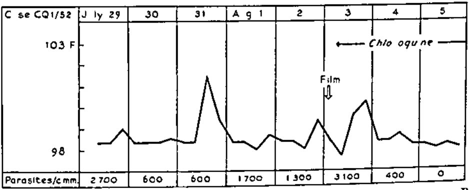
LV *Plasmodium malariae*



Original field

t—young trophozoite
s—mature schizont
e—polychromatic macrocytes one distorted with a cleft down the middle
n—neutrophil polymorph
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lm—large mononuclear leucocyte
p—blood platelets

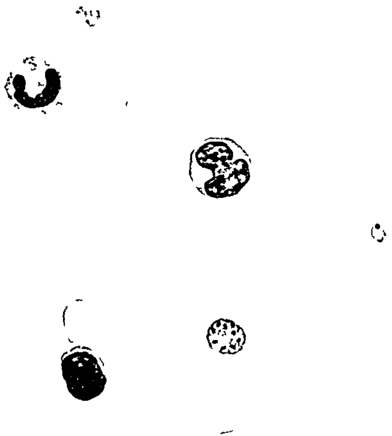
Clinical note—The patient an Indian woman 30 years old was admitted for confinement to the District Hospital Tampin on July 29th 1952 She said that she had been feverish for five days but she was afebrile on admission to hospital A month before she had had a similar



attack The spleen was not palpable There was a vague history of Paludrine treatment Blood examination revealed a low grade quartan infection with evidence of a macrocytic anaemia The haemoglobin reading was 40 per cent (Tallqvist) and a few normoblasts were present in the film The large size of the erythrocytes will be clear from a comparison of the plate with earlier plates all drawn to the same scale

Parasitological note—The film was taken on the morning of 3rd August when the patient though afebrile at the time was restless with a cold clammy skin The parasites present were young rings advanced trophozoites segmenters and gametocytes in the proportions 9 46 36 9 the rings and schizonts represented the rather straggling brood responsible for a mild paroxysm a few hours after the film was taken the older trophozoites arose from a generation which had segmented and produced a slight rise in temperature the day before (We are indebted to Dr J F B Edeson for the film and the clinical note)

LV *Plasmodium malariae*



QUARTAN MALARIA WITH MACROCYTIC ANAEMIA

The parasite density in quartan infections is usually low The count seldom exceeds 10 000 per c mm of blood and lower counts are usual Many patients are afebrile in the later stages of infection and the parasites are discovered by chance in the course of routine blood examination The plate shows a mature schizont and a young trophozoite a combination which is usual at the time of the paroxysm in patients with a quartan temperature The drawing was made from an Indian woman with a low grade infection She was pregnant and had been admitted to hospital for confinement The blood film revealed a rather marked macrocytosis which we suspect to have been related more to the pregnancy than to the malaria

LVI *Plasmodium ovale*

Colour correction—cytoplasm too red particularly that of the male gametocytes

The series of drawings, prepared from blood films taken from a patient infected with *P ovale* of West African origin illustrates some of the distinctive morphological features of the organism The films were three years old and somewhat faded a circumstance which explains the general pallor of the host cells

Trophozoites

Figs 2 4—Young ring forms Schuffner s dots appear very early (as they do with Malayan vivax)

Fig 5 —Young ' band form

Fig 6 —Double infection of cell a tendency for Schuffner s dots to collect around the periphery of the host cells is said to be a characteristic feature of ovale infections

Fig 8 —A compact trophozoite with little evidence of a vacuole—possibly a young gametocyte

Figs 9 12—Irregularity and elongation of the host cell are fairly common, sometimes with fimbriation of the edge

Fig 13 —Advanced trophozoite with a compact rounded form and a suggestion of early division in the nucleus

Schizonts

Figs 14 16—Early division of the nuclear chromatin

Figs 17 18—Advanced schizogony

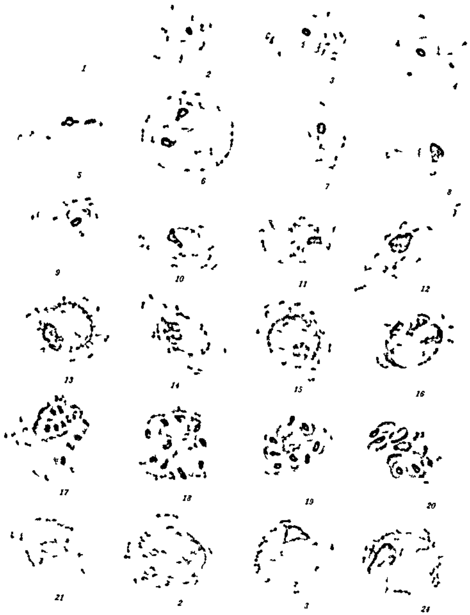
Figs 19 20—Mature segmenters

Gametocytes

Figs 21 22—Male gametocytes

Figs 23 24—Female gametocytes

LVI *Plasmodium ovale*



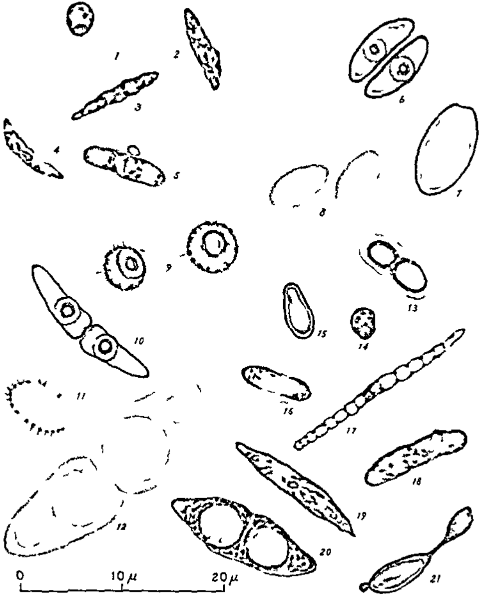
ASEXUAL BLOOD FORMS AND GAMETOCYTES OF A WEST AFRICAN STRAIN

LVII PUZZLES AND FALLACIES IN BLOOD EXAMINATION

Malaria diagnosis has many pitfalls for the unwary Yeasts fungi pollens and other cells of vegetable origin bacteria cell debris deposits of stain scratches on the slides, even the blood platelets may all give rise to puzzling appearances Their non malarial nature is usually obvious though they may sometimes lead to doubt or confusion in diagnosis The drawings reproduced in plates LVII to LIX were nearly all made from thin blood films taken for malaria diagnosis from patients in the wards of the General Hospital Kuala Lumpur

Figs 1 5—Bodies of unknown origin which may be mistaken for malaria parasites the intracellular body (1) vaguely resembles a quartan trophozoite and the elongated bodies (2 5) have a remote resemblance to falciparum gametocytes They are probably of vegetable origin

Figs 6 21—Various cells of unknown origin found in blood films from malaria patients Some are likely to be spores pollen grains or other vegetable cells carried over from the skin of the finger or they may be aerial contaminants which have fallen on the exposed films The bodies shown as figure 9 were floating in the immersion oil figures 11 14, 15 16 portray bodies present in films which had been left unstained on the laboratory bench for several days Few of these bodies are likely to confuse malaria diagnosis though some of them vaguely resemble crescents



SPORES POLLEN GRAINS AND OTHER CELLS OF VEGETABLE ORIGIN

Blood platelets—The blood platelets are usually unmistakable from their size their arrangement in clusters, their pale purple staining and their apparent woolly texture But abnormal forms may produce misleading appearances

Figs 1 and 2—Anomalous appearances of erythrocytes and platelets in a film taken on a slide which had been warmed to drive off the moisture and not allowed to cool sufficiently

Fig 3—Blood platelets superimposed on erythrocytes simulating degenerate parasites also a large elongated platelet

Algae—A deposit of green algae is not uncommon in phosphate buffer solution which has been kept for several months in the same bottle The algae do not deposit on thin blood films which are washed after staining under a brisk stream of water even though they are present in large numbers in the buffered stain diluent

Fig 4—Leishman stained algae from deposit in phosphate buffer solution

Devitrified glass slides—The surface defects in glass slides due to devitrification are likely to encourage a messy deposit of stain Devitrified slides should never be used for malaria diagnosis The surface bloom of devitrified slides is easily recognised if the dry slide is inspected at an angle to the light Devitrification is not uncommon in glass slides which have been dry stored for a long time in the tropics

Fig 5—Faint staining of the glass with the appearance of numerous pale areas, the film was taken on a devitrified slide

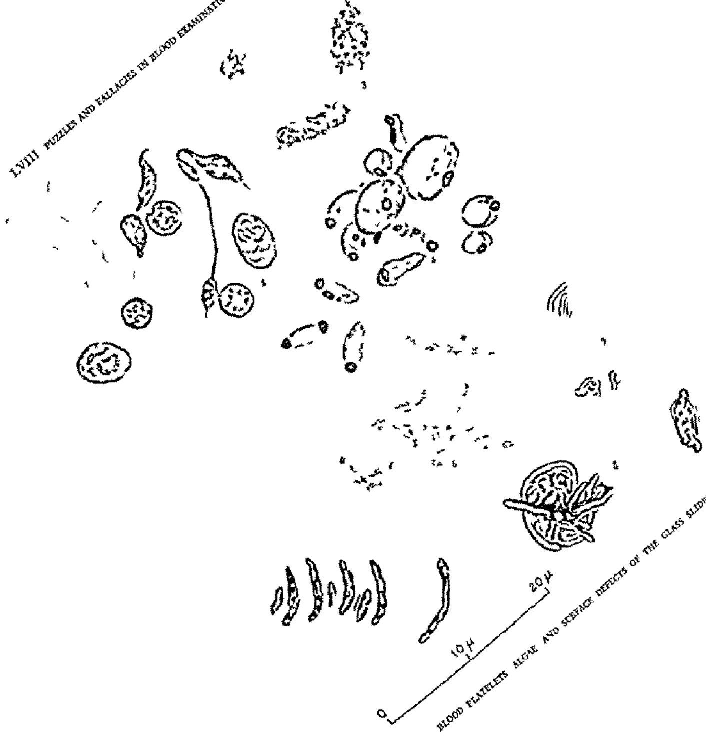
Fig 6—Another form of stain deposit in the surface defects of a devitrified slide

Scratched slides—Deposits of stain on scratched slides, though usually unmistakable are sometimes a cause of confusing artefacts Scratched slides should never be used for malaria diagnosis Some of the common appearances are portrayed in figures 7 9

Fig 7— Herring bone scratches which are easily recognised by the parallel arrangement

Fig 8—Scratches radiating from a circular erosion in the glass

Fig 9—Scratches on which erythrocytes have been superimposed



BLOOD PLATELETS ALGAE AND SURFACE DEFECTS OF THE GLASS SLIDES.

Bacteria—Various kinds of bacteria carried over from the skin sometimes appear in blood films, they may develop in films which have been left unstained and a rank growth of bacteria is not uncommon in films taken some hours after death

Fig 1—Clusters of cocci which may be mistaken for the merozoites of *P falciparum* the absence of cytoplasm and of the characteristic masses of falciparum pigment serves to identify them (cf plate X) The merozoites of *P falciparum* moreover, are likely to be seen in blood films only in heavy unmistakable infections

Fig 2—*Spore bearing bacilli*

Fig 3—Swollen micrococci which by some chance association with a red cell may simulate young malaria parasites These bodies were distributed in linear fashion along the line of spread of the film

Fungi—The hyphae or spores of contaminant fungi are not uncommon in blood films which have been left exposed to the air

Fig 4—Fungal spores in a film which had been left unstained for some days, the spores were associated with typical hyphae

Fig 6—These appearances found in a film containing the hyphae of a fungus simulate the marginal forms of *P falciparum* they are probably fragments of hyphae which have a chance attachment to the cells

Figs 7 and 9—Fungal hyphae and spores in an old blood film which had been re stained

Deposits of stain—Deposits of stain may sometimes produce puzzling appearances

Fig 5—Stain deposit simulating marginal parasites The deposits were dark almost black and readily recognised as artefacts by the absence of chromatin (cf plate XL) by their occurrence in groups of neighbouring cells and by their similar orientation in relation to the affected erythrocytes

Fig 8—Amorphous deposit of stain debris on the red cells, simulating degenerate parasites

LIX PUZZLES AND FALLACIES IN BLOOD EXAMINATION



BACTERIA FUNGI AND DEPOSITS OF STAIN

APPENDIX

APPENDIX

The morphology of malaria parasites damaged by drugs

Romanowsky (1891) was among the first to study the effect of treatment on the morphology of the malaria parasites in blood films Using the special stain which he had devised for malaria diagnosis he found that the tertian parasites show visual evidence of damage when the host is treated with quinine The nucleus disintegrates and the cytoplasm takes on a homogenous colouring breaks up and finally disappears Analogous changes during treatment with other drugs have since been described Mepacrine chloroquine proguanil and other schizontocidal drugs may so damage the parasites that the morphological features by which they are identified may be changed almost beyond recognition

Morphological description of the malaria parasites is normally based on the appearances of the undamaged organisms Microscopic diagnosis must take account also of their appearance in blood films from persons who have been treated Active remedies for malaria are available to-day in many a village shop and patients with fever often take a tablet or two before coming to hospital With modern drugs the dose will usually control the attack but it may be enough merely to damage the parasites and reduce their numbers Every worker in malaria knows the difficulty in diagnosis which may arise in these circumstances and for this reason a short account of the effect of drugs on the morphology of the parasites is included as an appendix to this Study

The selective action of drugs

The drugs which are useful as remedies for malaria seem to affect the parasites in different ways Some drugs have a lethal action on the nutritive phase of the asexual blood cycle they produce visible evidence of damage mainly in the trophozoites Quinine mepacrine chloroquine and amodiaquine are the important examples Drugs like proguanil and pyrimethamine on the other hand appear to act on the reproductive phase they disorganise nuclear division and destroy the schizonts The table below summarises these broad distinctions

MORPHOLOGICAL CHANGES IN THE ERYTHROCYTIC MALARIA PARASITES DURING TREATMENT

	Drug	Phase mainly affected	Visual evidence of damage
A	Mepacrine Chloroquine* Amodiaquine	Trophozoites	Growth retarded or arrested Rings contracted to mere blobs or thinned and expanded Cytoplasm ragged or fragmented Pigment granules clumped Few parasites able to complete development when the drugs are given early in the cycle
B	Quinine	Trophozoites	Similar changes but little effect on pigment
C	Proguanil Pyrimethamine	Schizonts	Nuclear division disrupted Degenerative changes in chromatin and cytoplasm Few parasites able to complete the cycle
D	Pamaquin	Gametocytes	Marked effect on viability with little visual evidence of damage

* Al p d cl mp g n, t trus of th p gm t in h gam tocytes with n ff n h bili y

The visual signs of degeneration are varied The cytoplasm may contract—with a loss of the vacuole in the ring forms—and appear dense and dark staining or it may be vacuolated or fragmentary and pale The nuclear chromatin may lose its normal sharp definition swell up and disintegrate When the degeneration is advanced the parasites may be represented merely by dissociated fragments of cytoplasm and chromatin and a few granules or clumps of pigment

Quinine

Quinine acts on the amoebae of malaria in those phases of its life cycle which are occupied in nutrition and development when however the transformation of haemoglobin into black pigment is arrested and in consequence the nutritive activity ceases and the reproductive phase begins then against this latter process quinine is of no avail This view advanced by Marchiafava and Bignami (1894) seems to have been generally accepted. The lethal effects of quinine fall mainly on the young parasites before the nucleus starts to divide many of the trophozoites cease to grow and few complete the cycle This inhibition of growth can usually be demonstrated in serial films but the morphological evidence of damage is

curiously variable some parasites still have a normal appearance after twenty four hours exposure to the drug while others show a marked degeneration in the cytoplasm and nuclear chromatin

The visual evidence of damage to the parasites during quinine treatment are seldom very marked in *P falciparum* The ring forms are usually recognisable until they disappear from blood films some are little changed in appearance others may be contracted to mere blobs with little distinction between the cytoplasm and the chromatin We have sometimes thought too that the larger trophozoites tend to stay in the peripheral blood to a rather later stage during quinine treatment particularly in heavy infections twenty four hours after the start of treatment the trophozoites may be larger than those normally seen in blood films and it is possible that quinine delays the normal recession of the organisms to the internal capillaries When the parasites are few and degenerate the presence of Maurer s dots in the host cells or of the characteristic falciparum pigment in the large mononuclears may be a useful aid to diagnosis Black (1946) has studied the effects of quinine on the later stages of the cycle When the parasites were exposed to quinine in cultures few grew beyond the presegmenting stage Thomson (1914) portrays quinine affected schizonts which have very few merozoites (plate XI) The gametocytes seen in blood films during quinine therapy retain their normal morphology

The effects of quinine in *P vivax* have been studied by Shute (1946) and by Mackerras and Ercole (1949*a*) Shute was unable to detect any gross morphological changes in the parasites up to four hours after the first dose of the drug But Mackerras and Ercole found marked changes after a more prolonged exposure the trophozoites were ragged in appearance with dispersed or fragmented cytoplasm others were dense and deep staining with no vacuole and little distinction between cytoplasm and chromatin. These small degenerate parasites were often seen on the margin of the cell Few parasites were able to *complete the cycle and the schizonts produced very few merozoites The gametocytes* as a rule show little morphological change yet despite the absence of gross evidence of damage they disappear from the peripheral blood the males within 2 3 days and the females a day or two later

Analogous changes during quinine treatment are seen in *P malariae* The trophozoites cease to grow or grow very slowly and many become ragged irregular or contracted Few complete development when the drug is given early in the cycle Trophozoites which remain at about the same size may sometimes be seen in blood films for several days Though degenerate the parasites can usually be identified by the usual morphological criteria

Mepacrine

The lethal effect of mepacrine on the malaria parasites like that of quinine seems to fall mainly in the early stages of the schizogonic cycle Trophozoites exposed to mepacrine are retarded in growth and degenerative changes appear in the cytoplasm and chromatin. Even more striking are the effects on the pigment of the older parasites The granules tend to coalesce into a few large clumps and a complete disappearance of the pigment due perhaps to be extrusion of these clumps from the organisms is not uncommon

The effects of mepacrine on *P falciparum* is apparent in blood films mainly in the changed morphology of the young trophozoites and gametocytes Some of the rings have a normal appearance others become ragged in outline or contract until they are little bigger than large cocci though diagnosis is usually possible until the parasites disappear from blood films Black (1946) from a study of *P falciparum* exposed to mepacrine in cultures states that development seldom passed beyond the amoeboid stage Sinton (1938) draws attention to a remarkable change in the appearance of the pigment of the gametocytes The pigment granules fuse together into a few irregular masses and sometimes the pigment disappears altogether There is no obvious effect on the cytoplasm and chromatin and the pigment free gametocytes have a normal appearance in other respects This effect of mepacrine on the pigment of the gametocytes seems to be related to the stage in the attack at which the drug is given The observations of Shute and Maryon (1952) suggest (*a*) that mature gametocytes in the peripheral blood are not affected by mepacrine (*b*) that most of the gametocytes reaching the peripheral blood have clumped pigment or no pigment at all when the drug is given before they are mature , and (*c*) that in relapses when there are rings and gametocytes in the peripheral blood a mixture of normal and pigment free gametocytes may be expected ten to fourteen days after the start of treatment a finding which Shute interprets to mean *that the drug* affects the young gametocytes without destroying them but does not affect those which are already mature and in the peripheral blood

The changes induced by mepacrine in *P vivax* have been studied particularly by Peter (1932) James (1934) Trager *et al* (1946) Mackerras and Ercole (1949*a*) and Shute (1952) Like quinine the drug acts mainly on the young trophozoites Degenerative changes in the cytoplasm of some of the ring forms are evident in a few hours growth is very slow and after twelve hours many of the parasites are reduced to mere wisps of cytoplasm and a diffuse detached mass of pigment. James (193) describes and illustrates the morphological changes in the parasites in blood films from a patient who had received a *single dose of* 600

milligrammes The cytoplasm appeared thin and lacerated and finally broke up into fragments and the normally compact nucleus opened out until it had the appearance of a loose tangle of broken fibres In the older parasites the pigment granules were aggregated into a few large clumps or else broke away from the parasite substance a clump could often be seen outside the parasite but still within the rim of the host cell Thus clumping of the pigment according to Trager *et al* (1946) may be seen within an hour of an intramuscular injection of 300 milligrammes and Shute (1952) has seen the same change within twenty minutes of an oral dose of 500 mgm Similar changes are reported by Mackerras and Ercole (1949) abnormal clumping in the pigment of the larger trophozoites began within two hours of the first dose of 500 milligrammes some rings appeared normal some already had pale and indistinct cytoplasm After six hours nearly all the rings were abnormal they had not grown and were often reduced to a mere band of cytoplasm and chromatin pressed against the margin of the cell resembling the marginal forms seen during quinine therapy All the schizonts were abnormal their nuclei were large and diffuse and the pigment was prematurely clumped or even extruded from the parasite A colour plate illustrates the appearance of the damaged trophozoites

The changes in *P malariae* during mepacrine treatment though much slower to appear are similar to the changes occurring in *P vivax* The growth of the young trophozoites is arrested or retarded and according to James (1932) similar degenerative changes appear in the cytoplasm and chromatin and in the form and distribution of the pigment

Chloroquine

Chloroquine and mepacrine are thought to act on the malaria parasites in much the same way Black (1946) found that the main action of chloroquine on *P falciparum* exposed to chloroquine in cultures was directed against the ring forms the cytoplasm disintegrated and disappeared leaving only the chromatin few trophozoites reached the presegmenting stage This is essentially the action of mepacrine No detailed study of the morphological changes induced by chloroquine in other species of Plasmodium infecting man is known to us our own limited observations suggest that they are very similar to those seen during mepacrine therapy

Amodiaquine (Camoquin)

From its chemical structure and action in malaria amodiaquine might be expected to resemble mepacrine and chloroquine in its effects on the malaria parasites but no detailed description of the effects of the drug on the morphology of the species infecting man is known to us Thompson (1948) states that the same morphological changes are produced by these drugs in *P lophurae* in chicks

Proguanil (Paludrine)

The lethal action of proguanil on the malaria parasites is thought to be directed mainly on the dividing nucleus The chromatin may fail to divide or it may divide once and then break up the schizonts being represented merely by fragments of chromatin and cytoplasm with scattered clumps of pigment

Proguanil seems to have little effect on the trophozoites of *P falciparum* Black (1946) found that the ring forms exposed to proguanil in cultures grow normally to the presegmenting stage thereafter the chromatin broke up without a true nuclear division Similar degenerative changes in the early schizonts from the peripheral blood and bone marrow have been reported by Fairley (1946) Gametocytes show no visible evidence of damage during proguanil therapy

The changes in *P vivax* have been described and illustrated by Mackerras and Ercole (1948) Nuclear division in *P vivax* is normally an orderly process The nucleus divides into two well defined masses which usually move apart before they finally divide into from eight to twenty or more discrete and well defined daughter nuclei The pigment granules do not begin to concentrate until nuclear division is fairly advanced The authors examined serial blood films from a patient with a vivax infection who was receiving 300 milligrammes of proguanil daily Most of the trophozoites grew normally differing little in appearance from healthy parasites of the same age But their development became disorderly when they reached the stage of nuclear division The chromatin might fail to divide or it might divide once and then swell and break up into diffuse masses When healthy developing schizonts were exposed to proguanil there was a similar disorganisation further division of the nucleus was arrested and the masses of chromatin became swollen and degenerate Normal merozoites were not seen nor did new rings appear in blood films The morphology of the gametocytes showed little change some gametocytes still appeared normal on the fifth day of treatment

The same authors describe the effects of proguanil on *P malariae* Large doses of the drug (1 0 gramme daily) were given to a patient with quartan malaria Again the drug seemed to disorganise the division of the nucleus though the morphological changes were slow to appear After 28 hours the large trophozoites and gametocytes had a normal appearance but the schizonts showed nuclear changes analogous to

those appearing in P vivax under the influence of proguanil The chromatin masses were swollen and distorted and apparently in the process of disintegration On the ring forms the drug seemed to have little effect other than a slowing down of growth in a second case observed the development of the rings proceeded slowly but otherwise normally for 72 hours until the organisms were finally destroyed at the schizont stage The gametocytes apparently unaffected at first were degenerate before they finally disappeared from blood films but whether this change was due to the drug or to the ageing of the organisms was not clear

Pyrimethamine (*Daraprim*)

The clinical and pharmacological similarity of pyrimethamine and proguanil suggests that their action on the parasites is likely to be the same The observations of June Thurston (1953) on the effect of these drugs on *P berghei* in mice lends support to this inference Pyrimethamine and proguanil attacked mainly the early schizonts causing a disintegration of the nuclei Mepacrine and chloroquine on the other hand produced a degeneration of the cytoplasm before the nuclei were visibly affected The only observation known to us on the morphology of the parasites during pyrimethamine treatment in human malaria is that of Shute and Maryon (1954) who were unable to detect any obvious change in the appearance of falciparum gametocytes

Pamaquin

Pamaquin and its more recent homologues are important malaria remedies mainly because of their lethal action on the gametocytes of *P falciparum* and on the exo-erythrocytic forms of *P vivax* They are seldom given alone as a remedy for acute malaria and their lethal action on the schizogonic forms in the blood has an academic rather than a clinical interest

The gametocytes of *P falciparum* lose their capacity to infect mosquitoes within a few hours when they are exposed to pamaquin even when the dose given to the host is very small but the morphological changes accompanying this striking loss of infectivity are slight Barber Komp and Newman (1929) and Shute and Maryon (1948) found no visible evidence of damage which they could definitely ascribe to the effects of the drug

The changes in *P vivax* during pamaquin therapy have been described and illustrated in a colour plate by Mackerras and Ercole (1949) who suggest tha the drug is a general protoplasmic poison affecting the erythrocytic organisms at all stages The ring forms and amoeboids showed degenerative changes in the cytoplasm and chromatin and the schizonts if not killed outright tended to round up and divide while still very small Sometimes only nuclear fragments and a vague suggestion of cytoplasm were left in cells stippled with Schuffner s dots

References

BARBER M A KOMP W H W AND NEWMAN B M (1929) The effect of small doses of plasmochin on the viability of gametocytes of malaria as measured by mosquito infection experiments *Pub Hlth Rep Wash* 44 1409

BLACK R H (1946) The effect of anti malarial drugs on *Plasmodium falciparum* (New Guinea strains) developing *in vitro* *Trans R Soc trop Med Hyg* 40 163

FAIRLEY N H (1946) Researches on Paludrine (M 4888) in malaria an experimental investigation undertaken by the L H Q Medical Research Unit (A I F) Cairns Australia *Ibid* 40 105

JAMES S P (1932) The direct effect of atebrin on the parasites of benign tertian and quartan malaria *Ibid* 28 3

MACKERRAS M J AND ERCOLE Q N (1948) Observations on the action of Paludrine on malarial parasites *Ibid* 41 365

— — (1949a) Some observations on the action of quinin atebrin and plasmoquine on *Plasmodium vivax* *Ibid* 4 443

— — (1949b) Observations on the action of quinine atebrin and plasmoquine on the gametocytes of *Plasmodium falciparum* *Ibid* 4? 455

MARCHIAFAVA E AND BIGNAMI A (1894) (ref p 3)

PETER F M (1932) Ueber die Wirkung des Atebrin gegen natürliche Malariainfektion *Deut Med Woch* 58 533 (Abstract *Trop Dis Bull* 193 29 705)

ROMANOWSKY (1891) Cited by Marchiafava and Bignami 1894

SHUTE P G (1946) Latency and long term relapses in benign tertian malaria *Trans R Soc trop Med Hyg* 40 189

—— AND MARYON M (1948) The gametocytocidal action of Paludrine upon infections of *Plasmodium fal iparum* *Parasitolo y* 38 264

—— —— (195) A study of human malaria oocysts as an aid to species diagnosis *Trans R Soc trop Med Hyg* 46 275

—— —— (1954) The effect of pyrimethamine (Daraprim) on the gametocytes and oocysts of *Plas nod um fal parum* and *Plasmod um vivax* *Ibid* 48 50

THURSTON JUNE P (1953) The morphology of *Plasmodium berghei* before and after treatment w th drugs *Ibid* 47 248

THOMPSON P E (1948) On the ability of *Plasmodium lophurae* to acquire resistance to chlorguanide Camoquin and chloroquine *J infe t Dis* 83 250

SINTON J A (1938) Action of atebrin upon the gametocytes (cres ents) of *Plasmod um falciparum* *T ans R Soc trop Med Hyg* 32 11

TRAGER W BANG F B AND HAIRSTON N G (1945) Relation of plasma level of atabrine to morphology and motility of *Plasmodium vi ax* *Proc So exp Biol Med N Y* 60 257 (Abstract *Trop Dis Bull* 1946 43 821)

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